Treatment of Antiviral Drugs in Wastewater Using Advanced Oxidation Processes – Ozonation of Oseltamivir Phosphate

By

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Abstract

While wastewater treatment facilities are designed to remove contaminants from water before it returns to the environment, unfortunately, not all of these facilities are effective in the removal of micropollutants (MPs). Antiviral drugs are a class of MPs that have become an area of concern due to their increased use and potential for negative impacts on the environment. An extensive literature review was undertaken to analyze the treatment methods, detection methods, and environmental fate of antiviral drugs to provide information on areas requiring further research. Treatment processes such as filtration, sedimentation, aerobic biological treatment, and anaerobic biological treatment were found to be generally unsuccessful for the removal of antiviral drugs. Advanced oxidation processes (AOPs) such as ozonation, UV/persulfate, and electro-oxidation processes showed significantly more promising results in the removal of antiviral drugs in water. However, there is a lack of some crucial information on AOPs treatment of antiviral drugs which including the disinfection byproducts (DBPs) that are produced which have the potential to possess toxic qualities as well. The environmental fate of antiviral drugs and their DBPs is under-researched with little information known on their properties such as adsorption, infiltration, or solubility, leaving a potential for accumulation that is not well understood. The effects of antiviral drugs on a variety of species is also unknown, with very few studies examining even the most directly affected aquatic wildlife.

Bench-scale studies examined the treatment of oseltamivir phosphate (OSP) using ozone (O_3) as the oxidant. Batch experiments were done to determine the first and second-order rate constants of the reaction between O_3 and OSP and ozone was found to be effective for the removal of OSP in buffered water (H_2O) with >99% removal of OSP. The impact of pH and various ionic species commonly found in wastewater were examined for effects on the rate of

degradation, both of which resulted in either minor or no change to the reaction rate. The ozonation of OSP in secondary effluent (SE) was required to prove effectiveness of the ozonation in real-life matrices and was able to remove >99% of the oseltamivir within 30 seconds with a 10:1 molar ratio of O_3 to OSP. Thirteen DBPs resulting from the ozonation of OSP were monitored with respect to time and structures were predicted, these were characterized into categories of increasing concentration with respect to ozone exposure, decreasing concentration, and unchanging concentration. Finally, the acute toxicity towards *V. fischeri* and the genotoxicity of both treated and untreated OSP samples were monitored; these tests showed that both ozonated and non-ozonated OSP samples in buffered and real wastewater matrices did not result in an increase in toxicity. This study overall suggests that ozonation has the potential to be a significant improvement for the treatment of OSP in sewage treatment plants.

Preface

The research described in this thesis is an original work by Shawn Jansen van Beek. The thesis was written and performed under the supervision of Dr. Mohamed Gamal El-Din in the Department of Civil and Environmental Engineering at the University of Alberta. The thesis is designed as a paper-format with the chapters 2 & 3 representing stand-alone papers that have been or will be submitted for publication. I conducted the experimental work and prepared the manuscript with the help of research assistants and post-doctoral fellows in our research group.

Dedication

To those who showed me the value of our world

To those who emphasized its frailty

To those who taught me to do my part

And those who will live here after me

Acknowledgements

All of the work presented in this thesis was done under the supervision of Dr. Mohamed Gamal El-Din to whom I am forever grateful. I would like to emphasize his guidance, willingness to pass on knowledge, and ability to improve others by constantly challenging and motivating me. I am also immensely grateful to Dr. Pamela Chelme-Ayala who was always there for me to discuss findings, question my assumptions, and plan my work during a time of much uncertainty. I would like to sincerely thank Dr. Zuo Tong How and Dr. Pamela Chelme-Ayala for lending me their insight in both assisting me with data analysis as well as reviewing and commenting on multiple edits of this thesis and the multitude of presentations throughout my research. I would also like to thank Miss Jia Li, Dr. Lingling Yang, and Dr. Mirna Alameddine for their contributions to the toxicity analysis and guidance on working with ozone. I would also like to acknowledge the financial support received from the Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development (CRD) Program and EPCOR Water Services.

I would also like to take that time to acknowledge those who gave me strength from outside the university setting. I would like to thank my family for simultaneously motivating me while keeping me grounded to the world outside of research. I want to acknowledge my friends who provided me with a setting to talk about my work and guide me through periods of doubt in a time where gatherings were incredibly difficult. And I would like to thank my partner for giving me strength in my lowest times, listening to my rambling, and pushing me to complete one of the most challenging tasks life has thrown my way. I sincerely thank and acknowledge that all of this work couldn't have happened without all of their loving support.

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Abbreviations

ACV	Acyclovir
AOP	Advanced oxidation processes
COD	Chemical oxygen demand
COFA	N-(4-carbamoyl-2-imino-5-oxoimidazolidin) – formamido-N-methoxyacetic acid
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DBP	Disinfection byproduct
EDC	Endocrine disruptor compound
ESI	Electrospray ionization
FVP	Favipiravir
G	Growth factor
HBV	Hepatitis B virus
HCQ	Hydroxychloroquine
HESI	Heated electrospray ionization
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
HRT	Hydraulic retention time
IBF	Ibuprofen
IF	Induction factor
LC	Liquid chromatography
LOQ	Limit of quantification
MAD	Mesophilic anaerobic digestion
MFC	Microbial fuel cell
MP	Micropollutant
MS	Mass spectrometry
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NPX	Naproxen

OC	Oseltamivir carboxylate
OD	Optical density
ODFW	Ozone demand-free water
OSP	Oseltamivir phosphate
PAC	Powdered activated carbon
PCP	Personal care product
RNA	Ribonucleic acid
SAF-MBR	Staged anaerobic fluidized membrane bioreactor
SE	Secondary effluent
SPE	Solid phase extraction
SPME	Solid phase microextraction
SRT	Solid retention time
TAD	Thermophilic anaerobic digestion
TBA	Tert-butyl alcohol
TCS	Triclosan
TDS	Total dissolved solids
TOC	Total organic carbon
TOF	Time of flight
TSS	Total suspended solids
UPLC	Ultra-high performance liquid chromatography
UV	Ultraviolet
WWTP	Wastewater treatment plant

Chapter 1: Introduction and Research Objectives

1.1 Background

Nearly a third of people on Earth do not have access to safely managed drinking water and only two fifths have access to safely managed sanitation services, resulting in billions of people who are left without their right to enjoy clean water, sanitation, and other related benefits (WWAP, 2019). The lack of clean water access is being aggravated by drastic population growth causing greater demand and thus greater competition in densely populated areas (Teklehaimanot et al., 2015; Vatankhah et al., 2019). The long-term effects of chemical pollution are largely unknown with respect to aquatic life or human health (Schwarzenbach et al., 2006). It should therefore come as no surprise that properly managed water treatment facilities are vital for successful society.

Micropollutants (MPs) are pollutants which are either natural or anthropogenic in nature that are present in trace concentrations in the environment. A steady increase in chemical pollution due to increasing use of chemical products by society has created a concern due to unknown effects on human and aquatic life (Margot et al., 2015). Types of micropollutants include pharmaceuticals, endocrine disruptor compounds (EDC), surfactants, personal care products (PCP), artificial hormones, industrial chemicals, steroids, pesticides, and many more (Sackaria et al., 2020). Micropollutants have been shown to bioaccumulate and thus have the potential to create health concern; EDCs are a category of MPs which have been linked to pancreatic effects such as decreased glucose levels and increased plasma insulin, affecting the ability for adult rodents to perform lactation or organogenesis, and impacting estrogen and androgen induction in the brain (Rubin, 2011). The removal of MPs via wastewater treatment

processes has also proven to be highly variable depending on the physico-chemical properties of the MPs, the treatment conditions, and the methods of treatment applied by the treatment plant (Luo et al. 2014).

Among the classes of MPs resides antiviral drugs, a class of compounds used for the treatment of viruses by aiding your body to fight against them. Antivirals are a broad classification of drugs which act in many different methods against viruses, these classifications include nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and viral entry inhibitors (De Clercq, 2004). There are also many viruses that antiviral compounds are currently being used to treat including hepatitis C virus, human cytomegalovirus, herpes simplex virus, influenza, DNA virus infections, RNA virus infections, and arenavirus (De Clercq, 2013). Antiviral drugs have been shown to produce toxic effects on aquatic life; data suggests that the antiviral drug efavirenz may lead to increased risk of liver damage, functional organ loss, and declines in overall fish health in *Oreochromis Mossambicus* (Robson et al., 2017). Studies have also shown that byproducts produced from the degradation of antiviral drugs can be toxic, with a metabolite of the drug acyclovir showing toxicity towards algae and *Vibrio fischeri* (Schutler-Vorberg et al., 2015; Prasse et al., 2012).

Oseltamivir phosphate is the commercially available pill for the drug oseltamivir, a commonly used and stockpiled drug that is used as treatment for seasonal and pandemic influenza internationally (Jefferson et al., 2014). Oseltamivir has been detected in natural waters at relatively high concentrations, ranging from 100 – 159 ng L^{-1} (Goncalves et al., 2011; Soderstrom et al., 2009; Takanami et al., 2012). Studies have also suggested that oseltamivir may cause toxicological effects towards aquatic life; scientific results have shown the development of

resistant variants of viruses in mallard ducks due to oseltamivir exposure which has the potential to circulate through other wild birds (Gillman, 2016; Gillman et al., 2015, Järhult, 2012).

Ozonation is a treatment method that is commonly used to disinfect wastewater and effectively remove organic compounds. Ozone as a treatment method for wastewater has increased in popularity due to two main reasons: decreased costs associated with ozone production, and environmental advantages over chlorine disinfection (Rekhate et al., 2020). The powerful oxidation potential of ozone (2.07 V) allows it to degrade pollutants via two mechanisms (i) direct oxidation involving attack by molecular ozone; (ii) indirect oxidation which utilizes hydroxyl radicals ($^{\bullet}OH$) generated during decomposition or reactions with other species (Rekhate et al., 2020).

1.2 Research Scope and Hypothesis

The purpose of this thesis is to pursue the advancement of knowledge in relation to antiviral drugs present in wastewater treatment. The research within seeks to develop insight on three different factors associated with this issue: ozonation treatment processes to remove the drugs, byproducts associated with degradation, and the results from the treatment processes. The experiments were performed at bench scale levels using both buffered water and real wastewater matrices to determine the effectiveness of the treatment methods and how they may operate in real world scenarios. The following hypotheses laid a framework for this research and provided direction for the studies:

1) Ozonation as a tertiary treatment process for the removal of oseltamivir from water and wastewater can effectively remove the pollutant efficiently.

2) The ability for ozone to degrade oseltamivir will differ depending on the conditions of the solute present, this includes how much of the oseltamivir will remain and the kinetics of the reaction.
3) The ozonation of oseltamivir will produce a set of byproducts, these will need to be further analyzed to determine potential risks associated with their formation.

1.3 Research Objectives

This thesis sought to understand and improve upon the removal of antiviral drugs from wastewater systems via ozone treatment processes. The initial goal was to identify and critically review gaps in knowledge related to the treatment of antivirals. Secondly, the research and experimentation discussed throughout was conducted to aid in solving the problem related to the release and abundance of the antiviral drug oseltamivir in the environment. Bench-scale studies were conducted to analyze the treatment and outcomes from treatment of the ozonation of oseltamivir.

1) Ozonation of oseltamivir in bench-scale systems with the goal of determining the effectiveness of ozone treatment and the kinetic data related to the reaction.
2) The effects of changing wastewater properties that impact the ozone degradation kinetics of oseltamivir
3) The analysis of disinfection byproducts (DBP) produced during ozonation of oseltamivir and examination of toxicological consequences related to the formation of said byproducts.

1.4 Thesis Outline

This thesis has been set into four chapters which were organized logically based on stages of research required to progress into the subsequent chapter.

- Chapter 1 is used as a general background on the environmental health issues related with the release of insufficiently treated water in relation to micropollutants and where antiviral drugs fit into this problem. It outlines the growing problem of antiviral drugs due to increased usage and insufficient treatment, as well as issues related to their release. The first chapter also describes the research objectives, hypotheses, and organization of the thesis.
- Chapter 2 is an extensive literature looking into antiviral drugs in relation to environmental health and wastewater treatment. The review goes into detail towards methods of detection, methods of wastewater treatment, environmental presence, and toxic effects of the drugs towards marine life.
- Chapter 3 explores the treatment of the antiviral drug oseltamivir via ozonation. The study examines the kinetics of the reaction between ozone and oseltamivir phosphate as well as factors that may affect the degradation in aqueous environments. The research advances to explore the disinfection products produced during the ozonation process and investigates potential risks associated with their formation.
- Chapter 4 summarizes the major findings and conclusions discovered during these works. It also provides recommendations for future work in this area based on the findings and limitations of current research.

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Chapter 2: Literature Review

2.1 Abstract

Antiviral drugs have emerged as pollutants of environmental concern due to their resistance to degradation and potential impacts on the environment. Antivirals specifically are of interest due to high consumption rates as a result of high prescription rates, influenza outbreak causing high peak emissions, and spatial diversity due to areas which report high human immunodeficiency virus risk. These pollutants enter water systems through excretion from humans, pharmaceutical industry waste, and domestic waste. Many antivirals have been detected in natural bodies of water around the world, often correlating with countries or areas with higher viral infection rates. Antiviral drugs have proven to be difficult to degrade via conventional wastewater treatment processes and are resistant to natural biodegradation, making them a high-risk category of pollutants. Treatments via advanced oxidation processes such as ozone are proving to be effective options for the removal of persistent pollutants, making this area of research promising. There is a critical lack of information on the removal methods and potential toxicological effects of antiviral drugs which require further research to develop a more complete understanding of the issues being faced. The objective of this review is to provide information and critical discourse on the current issues surrounding scientific practices related to the water treatment of antiviral drugs. This review analyzed the effectiveness of wastewater treatment methods towards antiviral drugs, and discovered shortcomings. Figure 2.1 presents an overview of antiviral drug removal and occurrence in the environment.

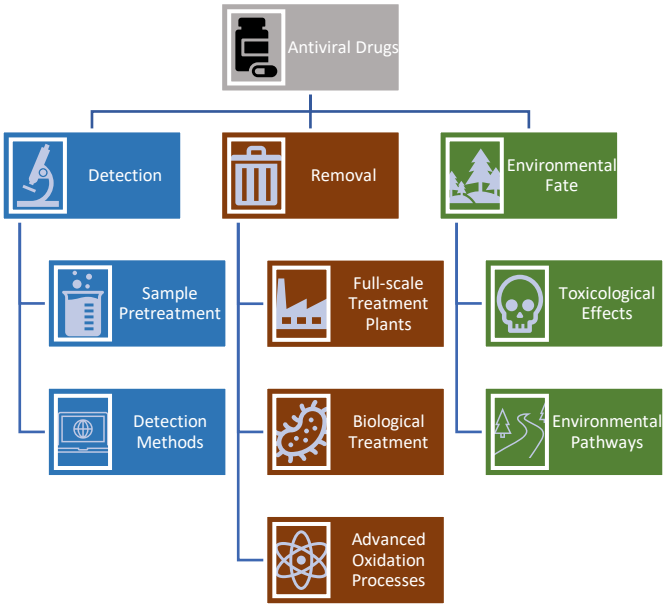


Figure 2. 1 Overview of antiviral drug removal, detection methods, and occurrence in the environment. The blue column highlights the sections surrounding analytical detection, the brown column highlights wastewater treatment, and the green column highlights environmental hazards

2.2 Introduction

Antiviral drugs have become an area of scientific interest due to the increased global usage and detection of many drugs in natural bodies of water (Prasse et al., 2010). Antiviral drugs are used to treat viruses worldwide, some of the most common are influenza, hepatitis A and B, and human immunodeficiency virus (HIV). The increase in antiviral use can be linked to increases in both treatment and occurrence of viruses. HIV specifically has become a global pandemic, with an estimated 38,000,000 people living with HIV, and 68% of adults receiving lifelong antiretroviral therapy, and 1,700,000 new cases in 2019 alone (UNESCO, 2019). The fact that many of these drugs being taken are entering natural water supplies creates concerns for both environmental and human health.

Antiviral drugs taken by patients are often not completely metabolized, and thus when the compounds are excreted, they enter water systems via sewer lines (Prasse et al., 2010). Many of these drugs are either partially removed or not removed at all during the conventional wastewater

treatment, and thus they are discharged and allowed to enter the environment (Prasse et al., 2010). Concentrations of antiviral drugs in natural water bodies have been documented worldwide and are suggested to be persistent in some cases (Jain et al., 2013). The persistence of these drugs in the environment and their responses to various environmental conditions are not well studied, making their potential impacts on the environment relatively unknown.

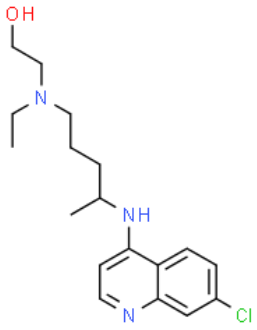
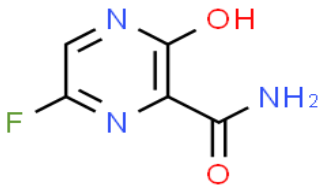
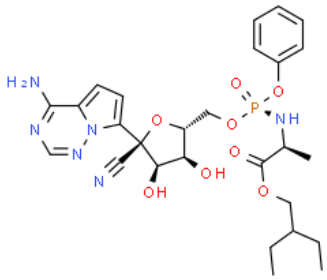
Environmental toxicity studies are under-researched for antiviral drugs, however, studies that have been done show significant toxic potential. Laboratory scale studies have shown toxic effects of antiviral drugs towards fish, mice, bacteria, daphnia, and L. sativa, suggesting possible toxic effects at elevated concentrations (Durjava et al., 2013; Oliveira et al., 2014; Prasse et al., 2012; Schutler-Vorberg et al., 2015; Feliciano et al., 2020). In addition to toxicological effects, the development of drug resistance is a constant fight in the medical industry, and exposure of antiviral drugs is believed to cause development of resistant genes (Jarhult et al., 2011).

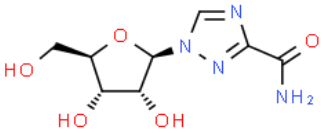
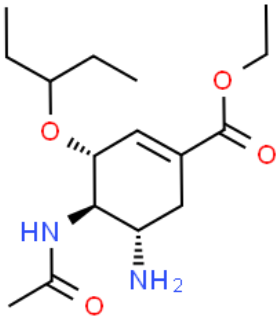
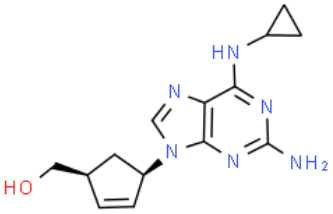
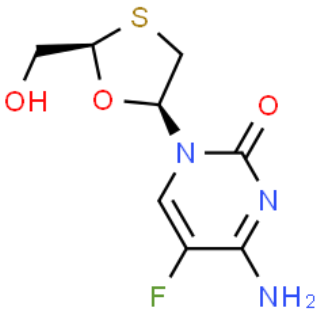
There is a vast variety of antiviral drugs as they are used to treat different viruses and have different mechanisms for inactivation. As a result of the large variety, antiviral drugs all react differently to treatment methods for their removal, however, a trend among many of them is an overall resistance to degradation by conventional treatments such as biological treatment, screening filtration, and adsorption (Prasse et al., 2010). Conventional treatments have shown highly varying results between drugs, with many persisting or even accumulating throughout the treatment process (Abafe et al., 2018; Prasse et al., 2010). On the other hand, advanced oxidation processes have proven to be significantly more successful in the degradation of antivirals, but detailed information on mechanisms and byproducts formation is lacking (Ternes et al., 2017).

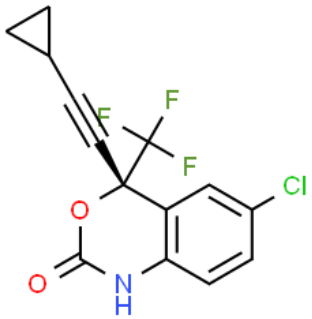
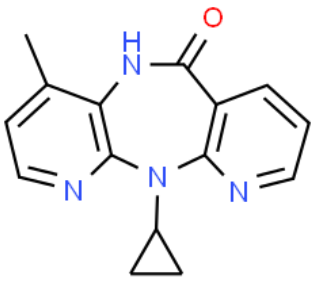
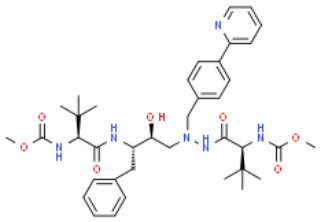
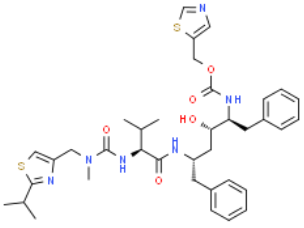
Research examining the removal of antiviral drugs from wastewater is lacking in many areas, the antiviral drugs examined in this review are outlined in Table 2.1. The goal of this

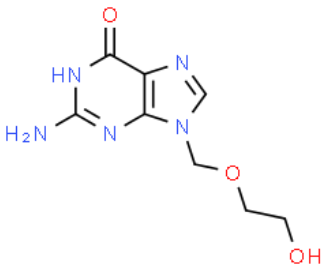
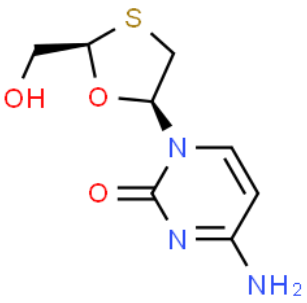
review is to provide the reader with sufficient background information on the available scientific literature around antivirals in natural and wastewater systems. Discussing the detection methods of detection, environmental fate, removal method, and toxicological effects of antiviral drugs will allow for a critical discourse on the potential impact of antiviral drugs.

Table 2. 1 Antiviral Drugs and Their Chemical Properties (*Data obtained from literature, National Library of Medicine, https://pubchem.ncbi.nlm.nih.gov/).

CAS no.	Name & Chemical Formula	Chemical Structure	Solubility* (25 °C)	pKa*	Density* (g/cm³)
118-42-3	Hydroxychloroquine $C_{18}H_{26}ClN_3O$		0.0261 mg/mL (ALOGPS est.)	9.67	1.2
259793-96-9	Favipiravir $C_5H_4FN_3O_2$		slightly soluble	5.1	1.6
1809249-37-3	Remdesivir $C_{27}H_{35}N_6O_8P$		0.339 mg/mL (ALOGPS est.)	10.23 and 0.65	1.5

CAS no.	Name & Chemical Formula	Chemical Structure	Solubility* (25 °C)	pKa*	Density* (g/cm³)
36791-04-5	Ribavirin $C_8H_{12}N_4O_5$		77 mg/mL (sulfate salt)	11.88 and -1.2	2.1
196618-13-0	Oseltamivir $C_{16}H_{28}N_2O_4$		soluble	14.03 and 9.31	1.1
136470-78-5	Abacavir $C_{14}H_{18}N_6O$		77 ng/mL (sulfate salt)	15.41 and 5.8	1.7
143491-57-0	Emtricitabine $C_8H_{10}FN_3O_3S$		112 mg/mL	14.29 and -3.1	1.8

CAS no.	Name & Chemical Formula	Chemical Structure	Solubility* (25 °C)	pKa*	Density* (g/cm³)
154598-52-4	Efavirenz $C_{14}H_9ClF_3NO_2$		.008855 mg/mL (ALOGPS est.)	12.52 and -1.5	1.5
129618-40-2	Nevirapine $C_{15}H_{14}N_4O$		0.0007046 mg/mL	10.37 and 5.06	1.4
198904-31-3	Atazanavir $C_{38}H_{52}N_6O_7$		slightly soluble	11.92 and 4.42	1.2
155213-67-5	Ritonavir $C_{37}H_{48}N_6O_5S_2$		insoluble	13.68 and 2.84	1.2

CAS no.	Name & Chemical Formula	Chemical Structure	Solubility* (25 °C)	pKa*	Density* (g/cm³)
59277-89-3	Acyclovir $C_8H_{11}N_5O_3$		1.41 mg/mL	9.35 and 2.52	1.8
134678-17-4	Lamivudine $C_8H_{11}N_3O_3S$		70 mg/mL	14.29 and -0.16	1.7

2.3 Types of Antiviral Drugs and Associated Health Risks

Antiviral drugs are used to treat many different types of viruses and work differently depending on the type of virus to be treated. As of 2016, 90 antiviral drugs which are categorized into 13 functional groups have been approved for the treatment of infectious disease, and thousands have been proposed in literature (De Clercq and Li, 2016). The drugs discussed here were chosen based on of how commonly they are used and information availability when relating to wastewater treatment.

Hydroxychloroquine (HCQ) is used to treat multiple types of diseases including both viruses such as HIV and malaria, and autoimmune diseases including rheumatoid arthritis, Sjörgen syndrome, and systemic lupus erythematosus (Van Loosdregt, 2013). The antiviral

mechanism of action for HCQ is believed to be the alkalinization of lysosomes and other intracellular acidic compartments, this inhibits the growth of intracellular pathogens (Plantone and Koudriavtseva, 2018). HCQ has shown good tolerability even during pregnancy, but some adverse effects have been reported including gastrointestinal and cutaneous manifestations and retinal, neuromuscular, and cardiac toxicities (Plantone and Koudriavtseva, 2018). An early clinical trial conducted on COVID-19 patients showed the drug has a significant effect on both clinical outcomes and viral clearance when compared to control groups (Gao, 2020).

Favipiravir (FVP) functions as a ribonucleic acid (RNA) polymerase inhibitor preventing the replication of several RNA viruses including arenaviruses, phleboviruses, hantaviruses, flaviviruses, enteroviruses, alphavirus, western equine encephalitis virus, paramyxovirus, respiratory syncytial virus, and noroviruses (Furuta et al., 2013). Favipiravir is introduced to cells where it is converted to an active phosphoribosylated form which is recognized as a substrate by viral RNA polymerase where it inhibits activity (Vafaei et al., 2019). The adverse effects of favipiravir have been shown to be relatively minor, with adverse reactions seen in roughly 20% of patients. The adverse effects included hyperuricemia, diarrhea, reduced neutrophil count, and transaminitis (Agrawal et al., 2020). Clinical trials have been initiated for the use of Favipiravir against the 2019 coronavirus which has pushed the drug into the academic spotlight (Dong et al., 2020).

Acyclovir (ACV) is an antiviral drug which works as an inhibitor for herpesvirus deoxyribonucleic acid (DNA) polymerase, this is accomplished by competing with deoxyguanosine triphosphate as a substrate for RNA polymerase, effectively stopping replication (Gnann et al., 1983). Acyclovir therapy has been associated with few adverse effects, however, renal disfunction, nephrotoxicity, agitation, hallucinations, disorientation, tremors, and

myoclonus have been reported in individuals (Gnann et al., 1992). Neurotoxicity has also been reported in patients as a side effect of intravenous acyclovir use with renal impairment (Chowdhury et al., 2016). Herpes simplex virus has also been shown to develop resistance to acyclovir through mutations in viral gene encoding of thymidine kinase. These acyclovir-resistant isolates have been shown to cause pneumonia, encephalitis, esophagitis, and mucocutaneous infections in immunocompromised patients (Gnann et al., 1992).

Remdesivir is an adenosine analog antiviral drug which was developed for the treatment of the Ebola virus infection. The antiviral drug has been applied against RNA virus families including filoviridae, paramyxoviridae, pneomoviridae, Ebola virus, respiratory syncytial virus, Hendra virus, and coronaviruses as an RNA polymerase inhibitor (Vafaei et al., 2019). Both SARS-CoV-1 and SARS CoV-2 have developed resistance to the antiviral drug remdesivir, two different mutations have cause 2.4-fold and 5-fold reduced susceptibility to remdesivir (Jorgensen et al., 2020). Adverse effects have been shown in users of remdesivir, including phlebitis, constipation, headache, ecchymosis, nausea, and extremity pain; some less common side effects include hypertension and transaminase elevations (Jorgensen et al., 2020). Remdesivir has shown promise in studies for COVID-19 treatment showing potent blocking of virus infection at low concentrations and high specificity towards coronavirus cells with no apparent side effects (Wang et al., 2020).

Ribavirin is a guanosine analog which works against RNA and DNA viruses like hepatitis C and E by inhibiting RNA synthesis via depletion of guanosine triphosphate pools in monophosphate form (Vafaei et al., 2019). Ribavirin has shown clinical efficacy against both influenza A and B viruses, respiratory syncytial virus, parainfluenza infections, and Lassa fever (Gilbert and Knight, 1986). Adverse effects have been shown in patients include decreased

hemoglobin levels, hypoxemia, psoriasis, eczema, and alopecia (Mistry et al., 2009; Chiou et al., 2005). Several viruses have developed resistance to ribavirin, including poliovirus, coronavirus, and influenza A, resulting in lower fidelity and higher specificity (Beaucourt and Vignuzzi, 2014). Due to ribavirin's effectiveness when applied synergistically with other treatment against SARS-CoV, it is believed that there is potential effectiveness against COVID-19, however, there is currently no data to support this claim (Zeng et al., 2020).

Oseltamivir is a prodrug of oseltamivir carboxylate which acts as a neuraminidase inhibitor, inhibiting the neuraminidase glycoprotein essential for replication of influenza A and B (McCellan & Perry, 2001). Studies have shown that oseltamivir follows a dose-dependant relationship for influenza patients and that five-day treatment periods demonstrated significant benefit in influenza patients (Arabi et al. 2020). Some adverse effects of oseltamivir that have been reported in humans are nausea, vomiting, diarrhea, abdominal pain, rash, swelling of the face or tongue, hypersensitivity, hepatitis, arrhythmia, dizziness, insomnia, vertigo, seizures confusion, delusions, diabetes, and fatigue (Tullu, 2009). Viral resistance to oseltamivir is rare due to the neuraminidase active site being highly conserved, allowing replication, however, an exception of this was the H1N1 virus which carried a resistant mutation in its genetic sequence.

Amantadine is a neuraminidase inhibitor which works by interfering with viral uncoating inside of the cell and blocking the M2 channel, preventing replication (Moscona, 2005). Originally, amantadine was developed as a treatment for influenza A, however, it was also adopted for treatment of Parkinson's disease (Hosenbocus and Chanal, 2013). Many countries now discourage the use of amantadine for seasonal influenza viruses due to development of high viral resistance, poor tolerability, and low effectiveness (Lehnert et al., 2016; Jefferson et al., 2006)

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which is used to mainly treat the HIV-1 virus (Adkins & Noble, 1998). Efavirenz either by itself or in cooperation with other synergistic drugs is commonly prescribed for HIV-1 treatment. This has led to the drug being seen commonly in countries like South Africa (Nannou et al., 2020). While efavirenz is generally considered safe, it displays adverse effects including cutaneous reactions, elevated plasma levels of liver transaminases, central nervous system symptoms, and erythema multiforme (Apostolova et al., 2017). Multiple mutations in HIV have been studied that cause resistance to efavirenz and other NNRTI's, these have been specifically located at the codons K101E and K103N as well as others (Parienti et al., 2004).

Nevirapine is a NNRTI of HIV-1 which is used often in combination therapy to treat HIV-1 infected individuals (Mirochnick et al. 2000). Studies have shown nevirapine is effective as both monotherapy and in combination with nucleoside analogues (Murphy and Montaner, 1996). Adverse effects that have been associated with nevirapine include hepatotoxicity, Johnson syndrome, and most commonly incidence of rash (Shubber et al., 2013). Similar to efavirenz, nevirapine suffers from mutations that cause resistance to NNRTI's, these mutations have been widely studied and multiple different mutations have been documented (Parienti et al., 2004).

Atazanavir is an azapeptide protease inhibitor which is most commonly used for the treatment of HIV-1 due to its specificity for HIV-1 protease (Goldsmith and Perry, 2003). It is often administered daily in combination with other HIV-1 treatment medications to boost effectiveness (Croom et al., 2009). Adverse effects associated with atazanavir treatment are most commonly nausea, jaundice, diarrhoea, and elevated total bilirubin (Croom et al., 2009). Resistance to atazanavir has been documented in both genotypic resistance and cross-resistance as a result of other protease inhibitor usage (Bentué-Ferrer et al., 2009).

Ritonavir is an artificial HIV protease inhibitor that has been used extensively in the treatment of HIV-1 for the last couple of decades (Hsu et al., 1998). Ritonavir can be taken by itself or in combination with other HIV-1 treatments and is often taken either daily or twice daily (Hsu et al., 1998). The most common side effects associated with ritonavir treatment are gastrointestinal effects such as diarrhea, nausea, and vomiting, with other more serious side effects such as pancreatitis, elevated transaminase levels, hypertriglyceridemia, and hypercholesterolemia (Chandwani and Shuter, 2008). Studies have suggested that ritonavir possess a high barrier to the development of resistance, and there has been no significant data that shows resistance mutations (Croxtall and Perry, 2010; Chandwani and Shuter, 2009).

Lamivudine is a deoxycytidine analog which works against hepatitis B virus (HBV). It has been shown to suppress HBV replication in chronic patients (Jarvis and Faulds 1999). Adverse effects associated with lamivudine are extremely limited, with no significant effects being noted in multiple studies examining immunosuppressed patients (Ehrhardt et al., 2015; Loomba et al., 2008; Katz et al., 2008). Lamivudine resistance in hepatitis B is significant, with a resistance rate of 24% after 1 year, and approximately 70% after 5 years; this can also lead to hepatitis flare ups (Sheng et al., 2011)

2.3 Analytical Methods for Antiviral Drug Determination and Quantification

The analytical determination of antiviral drugs in both natural and wastewater settings is a difficult process due to the large number of matrix interference effects and the extremely low (ng L^{-1}) concentration of the analytes. The analytical method should also be optimized to monitor metabolites and chemical transition products. The development of sample preparation and detection methods is a difficult and time-consuming task that requires extensive validation and calibration to ensure sufficient accuracy of the method. The selection of the analytical process

will depend on the target of the experiment, whether it be specific analyte detection or screening of many unknown analytes. However, most methods follow two main steps: samples clean-up and extraction in order to improve detection limits followed by analyte identification and quantification.

Sample preparation is a required step in the analyses of antiviral drugs in wastewater or natural water matrix due to significant matrix effects interfering with detection of the antiviral drugs. The most common sample preparation method is solid phase extraction (SPE) for isolation of pollutants and samples clean-up when looking at natural waters (Abafe et al., 2018; Aminot et al., 2015; Mosekiemang et al., 2019; Osunmakinde et al., 2013; Tong et al., 2011; Azuma et al., 2017; Prasse et al., 2010). Solid phase microextraction (SPME) is also a valid method of sample preparation for pharmaceuticals with advantages over SPE including lower sample volume requirement, no solvent requirement, higher enrichment factors, and ease of automation (Osunmakinde et al., 2013). Basic glass-fibre filtration is also used to prepare samples for analysis by removing large particulate matter (Azuma et al., 2017; Kovalova et al., 2012; Helbling et al., 2010).

Detection and quantification are conducted almost exclusively via Liquid chromatography coupled with mass spectrometry (LC-MS) with an electrospray ionization (ESI) source. This method allows for efficient separation of organic compounds via liquid chromatography providing more accurate quantification and detection of trace pharmaceuticals. A common mass spectrometric detection type is triple quadrupole which allows for detection of both parent and daughter ions, providing more information on the structure of the compound detected (Abafe et al., 2018; Aminot et al., 2015; Mosekiemang et al., 2019; Kovalova et al., 2012). Other mass spectrometric types include ion trap-Orbitrap (Helbling et al., 2010) and time

of flight (TOF) which can provide higher levels of precision compared to quadrupole instruments.

Accurate quantification of metabolites of many common pharmaceuticals and their degradation products can be difficult to quantify due to the lack of analytical standards. Common transformation products of antiviral drugs such as oseltamivir carboxylate and carboxy-acyclovir can be formed in humans during metabolism or during biological treatment processes. In some cases, these drugs can be directly acquired or synthesized in laboratory settings allowing them to be directly analyzed (Kovalova et al., 2012; Funke et al., 2016). When these products are not available, bench scale biotransformation systems such as activated sludge can be used to convert the drugs before testing (Helbling et al., 2010). Table 2.2 presents a summary of the analytical methods and detection limits for antiviral drugs.

Table 2. 2 Analytical Methods and Limits of Detection or Quantification for Analytical Drugs.

Antiviral	Detection Method	Limit of Detection	Limit of Quantification	Pre-Treatment	Matrix	Source
Oseltamivir	HPLC-ESI-MS		1 µg/L	N/A	N/A	Helbling et al. (2010)
	HPLC-MS/MS		5 ng/L	Filtration, SPE	Natural water	Kovalova et al. (2013)
	HPLC-ESI-MS/MS		0.2 - 1 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	LC-ESI-MS/MS		0.3 ng/L	Filtration, SPE	Natural water	Azuma et al. (2017)
	HPLC-ESI-HRMS		10 ng/L	SPE	Wastewater	Singer et al. (2016)
Oseltamivir	HPLC-MS/MS		25 ng/L	Filtration, SPE	Nanopure water	Kovalova et al. (2013)
carboxylate	HPLC-ESI-MS/MS		0.2 - 1 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	LC-ESI-MS/MS		0.6 ng/L	Filtration, SPE	Natural water	Azuma et al. (2017)
	HPLC-ESI-HRMS		10 ng/L	SPE	Wastewater	Singer et al. (2016)
	UPLC-ESI-MS/MS		4 - 6 ng/L	Filtration, Acidification, SPE	Wastewater	Ghosh et al. (2010)
Ritonavir	HPLC-MS/MS		1 ng/L	Filtration, SPE	Nanopure water	Kovalova et al. (2013)
	UPLC-ESI-MS/MS		0.787 ng/mL	SPE	Natural water	Mosekiemang et al. (2019)
	HPLC-ESI-MS/MS	3.0 ng/L		Filtration, Acidification, SPE	Natural water	Aminot et al. (2015)
	LC-HESI-MS/MS	5 ng/L	16 ng/L	SPE	Natural water	Abafe et al. (2018)
	UPLC-ESI-MS/MS	20 ng/L		Filtration, Acidification, SPE	Wastewater	Margot et al. (2013)
	UHPLC-HESI-MS	98 ng/L	297 ng/L	SPE	Natural water	Mhuka et al. (2020)
	HPLC-ESI-HRMS		50 ng/L	SPE	Wastewater	Singer et al. (2016)
Abacavir	HPLC-ESI-MS/MS		0.2 - 1 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)

	HPLC-ESI-MS/MS	0.2 ng/L		Filtration, Acidification, SPE	Natural water	Aminot et al. (2015)
	LC-HESI-MS/MS	4 ng/L	15 ng/L	SPE	Natural water	Abafe et al. (2018)
	LC-ESI-MS/MS		5 - 20 ng/L	Filtration	Surface and drinking water	Funke et al. (2016)
Acyclovir	HPLC-MS/MS		1 - 5 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	LC-ESI-MS/MS		5 - 20 ng/L	Filtration	Surface and drinking water	Funke et al. (2016)
Lamivudine	HPLC-ESI-MS/MS		10 - 100 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	UPLC-ESI-MS/MS		0.661 ng/mL	SPE (for surface water)	Natural water	Mosekiemang et al. (2019)
	HPLC-ESI-MS/MS	0.2 ng/L		Filtration, Acidification, SPE	Natural water	Aminot et al. (2015)
	LC-HESI-MS/MS	20 ng/L	65 ng/L	SPE	Natural water	Abafe et al. (2018)
	UHPLC-HESI-MS	4.91 µg/L	14.9 µg/L	SPE	Natural water	Mhuka et al. (2020)
	LC-ESI-MS/MS		5 - 50 ng/L	Filtration	Surface and drinking water	Funke et al. (2016)
Nevirapine	HPLC-ESI-MS/MS		1 - 5 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	UPLC-ESI-MS/MS		0.425 ng/mL	SPE (for surface water)	Natural water	Mosekiemang et al. (2019)
	HPLC-ESI-MS/MS	0.3 ng/L		Filtration, Acidification, SPE	Natural water	Aminot et al. (2015)
	LC-HESI-MS/MS	6 ng/L	20 ng/L	SPE	Natural water	Abafe et al. (2018)
	UHPLC-HESI-MS	11 ng/L	33 ng/L	SPE	Natural water	Mhuka et al. (2020)
	GC-TOFMS	1.8 ng/L	6 ng/L	SPE	Wastewater	Schoeman et al. (2017)
Ribavirin	HPLC-ESI-MS/MS		4 - 20 ng/L	Filtration, Acidification, SPE	Natural water	Prasse et al. (2010)
	HPLC-ESI-HRMS		>1000 ng/L	SPE	Wastewater	Singer et al. (2016)
Amantadine	LC-ESI-MS/MS		0.2 ng/L	Filtration, SPE	Natural water	Azuma et al. (2017)
	UPLC-ESI-MS/MS		4 - 6 ng/L	Filtration, Acidification, SPE	Wastewater	Ghosh et al. (2010)
Favipiravir	LC-MS/MS		0.4 ng/L	Filtration, SPE	Natural water	Azuma et al. (2017)
Efavirenz	UPLC-ESI-MS/MS		0.596 ng/mL	SPE (for surface water)	Natural water	Mosekiemang et al. (2019)
	LC-HESI-MS/MS	9 ng/L	31 ng/L	SPE	Natural water	Abafe et al. (2018)
	UHPLC-HESI-MS	59 ng/L	179 ng/L	SPE	Natural water	Mhuka et al. (2020)
	GC-TOFMS	7.8 ng/L	25.9 ng/L	SPE	Wastewater	Schoeman et al. (2017)
Emtricitabine	UPLC-ESI-MS/MS		0.919 ng/mL	SPE (for surface water)	Natural water	Mosekiemang et al. (2019)
	LC-ESI-MS/MS		20 - 50 ng/L	Filtration	Surface and drinking water	Funke et al. (2016)
Atazanavir	LC-HESI-MS/MS	2 ng/L	12 ng/L	SPE	Natural water	Abafe et al. (2018)
	UHPLC-HESI-MS	95 ng/L	289 ng/L	SPE	Natural water	Mhuka et al. (2020)

2.4 Wastewater Treatment of Antiviral Drugs

The presence of antiviral drugs in wastewater treatment plants (WWTPs) globally depends on several factors, including location of the WWTP, medical requirements of the local population, the standard of living, and the government approval of certain pharmaceuticals. The

removal of antiviral drugs from human wastewater is a matter of extreme importance and is often overlooked during the testing and approval process of new drugs. The rigorous testing process of new pharmaceuticals fails to consider the potential effects of both the drug and its metabolites on other potentially effected species including aquatic and plant life. This lack of testing leaves the burden of testing on other researchers to ensure these new drugs have minimized environmental impact.

WWTP processes have many steps relating to the removal of contaminants often including aerobic biological digestion, anaerobic biological digestion, disinfection, and filtration, all of which contribute to the removal of organic compounds. It is important to understand the effectiveness of each process individually to effectively target and eliminate pollutants.

2.4.1 Full-Scale Wastewater Treatment Plant

The removal of antiviral from full-scale WWTPs is important because they are the barriers preventing the spread of these compounds to the environment (Luo et al., 2014). WWTPs generally follow four major steps in their process which include preliminary, primary, secondary, and tertiary treatment (Ranjit et al., 2021). Preliminary treatment methods are generally used to remove floating debris, while primary treatment is used to remove small inorganic matter as well as large settleable organic matter. Secondary or biological treatments are used to remove suspended solids and organic material, and tertiary treatment is employed to remove the total suspended solids (TSS), total dissolved solids (TDS), organic and inorganic matter, nutrients, and killing pathogens found in secondary effluent (Ranjit et al., 2021). Figure 2.2 summarizes the removal efficiencies of antiviral drug via conventional treatment. Conventional treatment here defined as processes trains that do not include advanced treatments such as ozone, peroxide, UV irradiation, and membrane filtration.

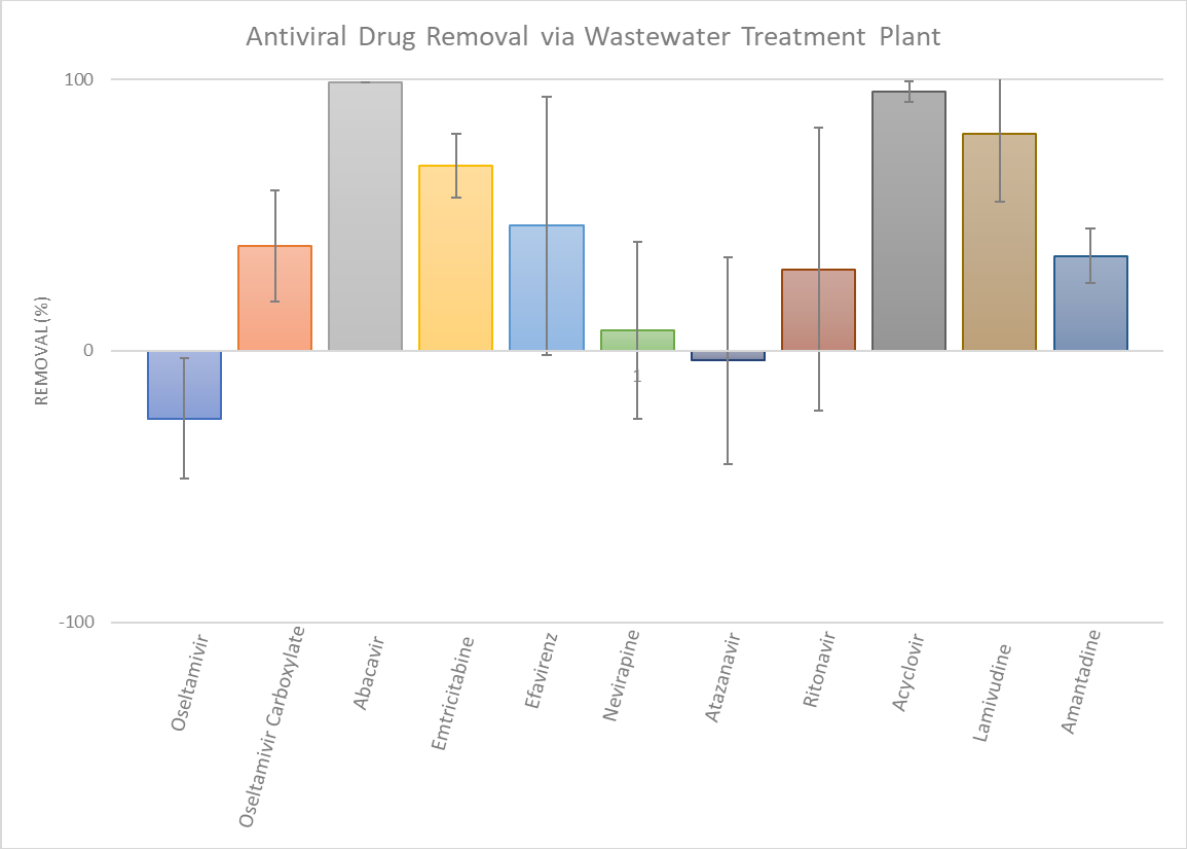


Figure 2. 2 Removal Efficiencies of Antiviral Drugs via Conventional Treatment with Standard Deviations (Gago-Ferrero et al., 2020; Schoeman et al., 2017; Funke et al., 2016; Mhuka et al., 2020; Abafe et al., 2018; Prasse et al., 2010; Kovalova et al., 2013; K'oreje et al., 2016).

The antiviral drug ribavirin was only discussed in one study examining the removal efficiency of two conventional WWTPs with aerobic chemical oxygen demand (COD) removal and combined anerobic denitrification and aerobic nitrification (Prasse et al., 2010). Because the ribavirin concentrations in the influent and effluent were below the LOQ of 4 ng L^{-1} in both separate locations, the removal efficiency could not be reported.

The removal of acyclovir was monitored in treatment plants consisting of mechanical treatment, biological nitrification/denitrification, and chemical phosphorus. The studies found removal efficiencies of 97 – 98% with an initial concentration of 1800 ng L^{-1} (Prasse et al., 2010)

and 91% with an initial concentration of 750 ng L^{-1} (Funke et al., 2016) in wastewater treatment plants. These results suggest that acyclovir is removed effectively by conventional treatment.

Removal of lamivudine was examined in treatment plants consisting of combined processes of primary treatment, biological treatment, and chlorination. One study examining the removal of lamivudine using aerobic treatment, anaerobic digestion, and chlorination found influents ranged in concentration from 840 – 2200 ng L^{-1} and effluents <130 ng L^{-1} suggesting efficient removal (Abafe et al., 2018). A study examining the removal of two treatment plants consisting of screening, grit removal tank, primary clarifier, and biological nitrification and denitrification found lamivudine was removed at >93% with influents ranging from 210 – 720 ng L^{-1} (Prasse et al., 2010). A study examining the Daspoort Wastewater Treatment Works found an influent concentration of 267 ng L^{-1} and an effluent of 28.08 ng L^{-1} for lamivudine, a roughly 90% removal rate. A study examining multiple different treatment processes found that conventional treatment consisting of mechanical treatment, biological nitrification and denitrification, and chemical phosphate removal only transformed lamivudine to its carboxylate form, while ozonated effluent was able to remove this carboxylate entirely (Funke et al., 2016). These studies suggest that lamivudine may not be properly eliminated through biological and mechanical treatment but transformed to the carboxylate salt, requiring further treatment such as ozonation to be removed (Prasse et al., 2010; Funke et al., 2016; Abafe et al., 2018; Mhuka et al., 2020).

Treatment plants consisting of primary mechanical treatment with secondary biological treatment were examined for removal of abacavir. A study examining the removal of abacavir in wastewater treatment plants consisting of aerobic treatment, anaerobic digestion, and chlorination found influent concentrations ranging from 3500 – 14000 ng L^{-1} with >99% removal

efficiency (Abafe et al., 2018). A European study examined the removal of abacavir from two treatment plants consisting of screening, grit removal tank, primary clarifier, and biological nitrification and denitrification found influent concentrations ranging from 21 – 81 ng L^{-1} with >99% removal efficiencies (Prasse et al., 2010). A study examining multiple wastewater treatment plants on abacavir removal found that conventional treatment consisting of mechanical treatment, biological nitrification and denitrification, and chemical phosphorus removal was only able to convert lamivudine to the carboxylate salt, but that further ozonation of the effluent was able to remove that carboxylate. These studies suggest that biological and mechanical treatment can transform abacavir to the carboxylate salt but are not sufficient to full breakdown the drug (Prasse et al., 2010; Funke et al., 2016; Abafe et al., 2018).

Conventional treatment methods were found to be ineffective in the removal of nevirapine . Treatment involving facilities running activated sludge and chlorine processes found very little removal ranging from 0 – 50% with influent concentrations ranging from 9 – 2800 ng L^{-1} (Abafe et al., 2018; Mhuka et al., 2020). Accumulation of nevirapine during treatment, with influent concentrations ranging from 5 – 200 ng L^{-1} and effluent concentrations ranging from 7 – 500 ng L^{-1} have also been reported in facilities consisting of biological and chlorine treatment, suggesting that conventional treatment is ineffective for removal of nevirapine and that these pollutants may bioaccumulate in sludge and sediment (Prasse et al., 2010; Schoeman et al., 2017). The authors state that it is unclear where this accumulation of nevirapine comes from, but it can be concluded that it is resistant to degradation by activated sludge and chlorine.

The removal of oseltamivir was assessed using conventional treatment, including mechanical treatment, activated sludge, and chemical phosphorus removal (Prasse et al., 2010; Kovalova et al., 2012). Both studies reported an accumulation of oseltamivir in the final effluent,

with influent concentrations ranging from <0.2 – 25 ng L^{-1} suggesting the resistance of oseltamivir to biological degradation and adsorbance; neither study probided a significant explanation for the cause of this increase. Oseltamivir carboxylate treatment was examined in two treatment facilities consisting of grit removal, primary clarification and biological nitrification/denitrification found an average 59% removal with influent concentrations ranging from 29 – 42 ng L^{-1} and effluent concentrations ranging from 12 - 17 ng L^{-1} (Prasse et al., 2010). Treatment of oseltamivir was also examined in two other facilities with primary and secondary treatment noting removal efficiency between 20 – 40%, another facility which used a tertiary ozone treatment and showed >90% removal (Ghosh et al., 2010). These studies suggest while oseltamivir carboxylate is resistant to biological treatment, however, advanced oxidation processes may be effective for degradation.

Amantadine was examined in three studies, all of which examined treatment plants consisting of processes including mechanical and activated sludge treatment; one study found amantadine in WWTP effluent ranging from 22 – 100 ng L^{-1}, another reported an influent concentration of 60 ng L^{-1} and an effluent of 40 ng L^{-1} in a treatment plant with primary sedimentation, activated sludge, biological nitrogen and phosphorus removal, and secondary sedimentation (Singer et al., 2016; Gago-Ferrero et al., 2020). Another which examined multiple treatment plants which reported roughly 10% removal during primary treatment consisting of screening and grit removal, 20 – 40% removal during secondary treatment consisting of aerobic and anaerobic biological nutrient removal, and >90% removal using ozonation as a tertiary treatment (Ghosh et al., 2010). These studies suggest that mechanical and biological treatment are insufficient for removing amantadine from wastewater.

Removal of emtricitabine was examined in treatment plants with process trains including mechanical primary treatment, activated sludge, and phosphorus removal; the study found moderate removal, with influent concentration of 0.33 μg L^{-1} and effluent 0.15 μg L^{-1} (Gago-Ferrero et al., 2020). Another study found emtricitabine was only partially removed and mostly converted to the carboxylate salt via mechanical treatment, biological nitrification and denitrification, and chemical phosphorus removal; however, ozonation and powdered activated carbon (PAC) treatment of effluent was able to effectively remove all of the emtricitabine and carboxylate salt (Funke et al., 2016). These studies indicate biological treatment is likely insufficient for complete removal and further tertiary treatment may be required.

Four studies examined the removal of atazanavir, all of which examining conventional processes with primary and secondary treatment and no chlorination. Atazanavir was detected in the effluent of wastewater treatment plants with concentrations ranging from 150 – 770 ng L^{-1} (Singer et al., 2016; Ibáñez et al., 2017). The removal of atazanavir was monitored in treatment facilities involving primary sedimentation, activated sludge treatment, biological nitrification and phosphorus removal, and secondary sedimentation showed removal efficiencies ranging from -25 – 50% with influent concentrations ranging from 20 – 1400 ng L^{-1} (Gago-Ferrero et al., 2020; Abafe et al., 2018). This suggests that atazanavir is inefficiently removed via conventional treatment processes.

Treatment of ritonavir in plants consisting of primary sedimentation, activated sludge process, and biological nitrification and phosphorus removal reported removal efficiencies ranging from 25 – 30% with influent concentrations ranging from 25 – 110 ng L^{-1} and effluent concentrations ranging from 15 – 90 ng L^{-1} (Margot et al., 2013; Gago-Ferrero et al., 2020). Treatment plants consisting of aerobic activated sludge nutrient removal, anaerobic digestion,

and chlorination found removal efficiencies ranging from 40 – 70% with influent concentrations ranging from 1600 – 3200 ng L^{-1} and effluent concentrations ranging from 460 – 1500 ng L^{-1} (Abafe et al., 2018). Based on this information, it is likely that ritonavir is removed partially by both biological and chlorination treatment, but further tertiary treatment is likely required for complete removal.

Efavirenz treatment was examined in three different studies, all of which reported quite different levels of removal. A study by Schoeman et al. (2017) found removal efficiencies ranging from 20 – 70% with influent concentrations ranging from 5000 – 14000 ng L^{-1}, however final effluent concentration remained constant. The study by Abafe et al. (2018) reported removal efficiencies ranging from roughly -50 – 50% with influent concentrations ranging from 24000 – 34000 ng L^{-1} and effluent concentrations ranging from 20000 – 34000 ng L^{-1}, providing no clear conclusion on removal. Both the Schoeman and Abafe study examined treatment plants consisting of activated sludge and chlorination process trains, the results however provide little information on what the mechanism of removal may be. The study by Mhuka et al. (2020) reported an influent concentration of 1171 ng L^{-1} and an effluent concentration of 1036 ng L^{-1}, however, did not report the process train used in the Daspoort Wastewater Treatment Works leaving a gap in required information. These studies leave a lot of research to be conducted to determine the removal mechanism of efavirenz and how effective conventional treatment is. Table 2. 3 summatizes the treatment processes for antivirals and relevant findings.

Table 2. 3 Treatment Processes for Antivirals and Relevant Findings.

Antiviral	Region	Treatment Process	Relevant Findings	Reference
Abacavir	Germany	Conventional treatment	>99% removal	Prasse et al. (2010)

	n/a	Electrochemical oxidation with Ti/SnO2-Sb anode	>97% removal in 10 minutes	Zhou et al. (2019)
	Germany	Conventional treatment	>99% removal	Funke et al. (2016)
	n/a	Ozone treatment	92% removal	Ternes et al. (2017)
	South Africa	Conventional treatment	75 - 100% removal	Abafe et al. (2018)
	South Korea	Staged anaerobic fluidized membrane bioreactor	62 - 100% removal	McCurry et al. (2014)
Acyclovir	Germany	Conventional treatment	97% removal	Prasse et al. (2010)
	Germany	Conventional treatment	91% removal	Funke et al. (2016)
	n/a	Ozone treatment	99% removal	Ternes et al. (2017)
	South Korea	Staged anaerobic fluidized membrane bioreactor	94 - 96% removal	McCurry et al. (2014)
	n/a	Ozone treatment	carboxy-acyclovir complete degradation, COFA formed	Schluter-Vorberg et al. (2015)
	n/a	Activated Sludge	Biodegradation half life 5.3 h, degradation rate constant 4.9 ± 0.1 L/gSS*d	Prasse et al. (2011)
	n/a	Ozone treatment	rate with molecular ozone 1.8x104 M-1s-1 for neutral form and 3.4x106 M-1s-1	Prasse et al. (2012)
	n/a	UV/H2O2	Rate constant (1.23±0.07)x109 M-1s-1	Russo et al. (2017)
	n/a	Membrane bioreactor + ozone	99% removal via MBR, further 99% removal via ozone	Mascolo et al. (2010)
Atazanavir	South Africa	Conventional treatment	-25 - 50% removal	Abafe et al. (2018)
	Switzerland	Conventional treatment	Detected in 5 of 6 samples at 150 - 770 ng/L	Singer et al. (2016)
	Athens	Conventional treatment	Detected in 7 of 7 effluent samples	Ibanez et al. (2017

	Athens	Conventional treatment	No removal	Gago-Ferrero et al. (2020)
Amantadine	Japan	Conventional treatment	7 - 13% removal primary treatment, 20 - 39% removal secondary treatment, 93% removal tertiary	Ghosh et al. (2010)
	Switzerland	Conventional treatment	Detected in 5 out of 6 samples from 22 - 100 ng/L	Singer et al. (2016)
	Athens	Conventional treatment	33% removal	Gago-Ferrero et al. (2020)
	n/a	Fenton + ultrasonic process	17.1% removal via fenton process, 30.5% removal via ultrasonic/H2O2, 65.6% removal combined	Zeng et al. (2015)
	n/a	Photocatalytic degradation	Degradation rate constant 6.31 x $10^{\wedge 9}$ $M^{\wedge -1}$ $s^{\wedge -1}$	An et al. (2015)
Favipiravir	Japan	Photodegradation and biodegradation	99% removla via photodegradation, persistent against biodegradation	Azuma et al. (2017)
Efavirenz	South Africa	Conventional treatment	-50 - 50% removal	Abafe et al. (2018)
	Germany	Conventional treatment	70% removal	Schoeman et al. (2017)
	Pretoria	Conventional treatment	12% removal	Mhuka et al. (2020)
Emtricitabine	Germany	Conventional treatment	74% removal	Funke et al. (2016)
	n/a	Ozone treatment	97% removal	Ternes et al. (2017)
	South Korea	Staged anaerobic fluidized membrane bioreactor	-63 - 83% removal	McCurry et al. (2014)
	Athens	Conventional treatment	54% removal	Gago-Ferrero et al. (2020)
Hydroxychloroquine	n/a	Electrochemical oxidation + UV/Sonication	Complete removal, improved efficiency when combined with UV and sonication	Bensalah et al. (2020)

	n/a	Photodegradation	Half life of 2.9 h	Dabic et al. (2019)
Lamivudine	Germany	Conventional treatment	>76 - >93% removal	Prasse et al. (2010)
	Germany	Conventional treatment	93% removal	Funke et al. (2016)
	n/a	Ozone treatment	94% removal	Ternes et al. (2017)
	South Africa	Conventional treatment	90 - 100% removal	Abafe et al. (2018)
	South Korea	Staged anaerobic fluidized membrane bioreactor	90 - 100% removal	McCurry et al. (2014)
	Pretoria	Conventional treatment	90% removal	Mhuka et al. (2020)
	n/a	Photocatalytic degradation with TiO2 catalyst	Degradation rate of 0.542 min^-1, complete degradation in 60 minutes	An et al. (2011)
	n/a	electrochemical oxidation with Ti/SnO2-Sb/Ce-PbO2 anode	Reaction rate ranging from 0.129 - 0.144 min^-1, removal rate ranging from 91.4 - 96.0%	Wang et al. (2019)
	n/a	UV/H2O2	95.56% removal	Feliciano et al. (2020)
Nevirapine	South Africa	Conventional treatment	0 - 50% removal	Abafe et al. (2018)
	Germany	Conventional treatment	Accumulation through process	Schoeman et al. (2017)
	South Africa	Chlorine treatment	Ineffective for removal	Wood et al. (2016)
	n/a	Photocatalytic degradation	68.5% removal maximum	Bhembe et al. (2020)
	Pretoria	Conventional treatment	Accumulation through process	Mhuka et al. (2020)
Oseltamivir	Germany	Conventional treatment	No removal	Prasse et al. (2010)
	Switzerland	Membrane bioreactor	-42 ± 149% removal	Kovalova et al. (2012)
	Switzerland	PAC, ozone, UV	>63% removal via PACm 40% removal via UV	Kovalova et al. (2013)

Oseltamivir carboxylate	n/a	biodegradation via bioplastic formulation	Persistence against biodegradation with >50% removal in 30 days	Accinelli et al. (2010)
	Switzerland	Ozone and hydroxyl radical	50% removal	Mestankova et al. (2012)
	Germany	Conventional treatment	59% removal	Prasse et al. (2010)
	Switzerland	Membrane bioreactor	18 ± 62% removal	Kovalova et al. (2012)
	Switzerland	PAC, ozone, UV	>36% removal via PAC, -2% removal via UV	Kovalova et al. (2013)
	Japan	Conventional treatment	>10% removal in primary treatment, 15 - 37% removal in secondary, 90% removal in tertiary	Ghosh et al. (2010)
Remdesivir	n/a	Photodegradation	No removal	Avataneo et al. (2020)
Ritonavir	Switzerland	Membrane bioreactor	78 ± 16% removal	Kovalova et al. (2012)
	Switzerland	PAC, ozone, UV	>87% removal via PAC	Kovalova et al. (2013)
	South Africa	Conventional treatment	40 - 70% removal	Abafe et al. (2018)
	Pretoria	Conventional treatment	-76% removal	Mhuka et al. (2020)
	Athens	Conventional treatment	16% removal	Gago-Ferrero et al. (2020)
	Switzerland	Conventional treatment, ozone, PAC-UV	WWTP removal 25%, ozone removal >78%, PAC-UF removal >56%	Margot et al. (2013)

2.4.2 Biological Treatment

Biological treatment is a common method of wastewater treatment due to its low cost, ease of application, and the abundance of pre-existing knowledge around its use. Biological treatment encompasses many types of treatment, such as maturation ponds and activated sludge, that all revolve around the use of microbial communities to remove contaminants from water. Many biological treatment processes such as activated sludge also incorporate the physical

process of adsorption which can work to remove micropollutants (Akhtar et al., 2016). These processes are often combined with other physical and chemical processes to improve their efficiency. Several different treatment methods can be classified as biological including activated sludge, anaerobic digestion, biological nitrification/denitrification, and others. Biological treatment is an essential process of wastewater micropollutant removal, however, critical information is still lacking such as the main drivers of biological removal and critical parameters such required retention times, nutrient requirements, and temperature, that affect degradation efficiency (Falas et al., 2016; Kanaujiya et al., 2019). This lack of critical information has resulted in a gap in knowledge of the overall effectiveness of biological treatment towards antiviral drugs.

Anaerobic digestion is an area of wastewater treatment that is gaining interest in academics due to its potential for energy production and storage via microbial fuel cell (MFC) technology (Wee Seow et al., 2016). Research examining the removal efficiency of certain abundant micropollutants via anaerobic digestion has accompanied MFC research, however, research examining antivirals specifically is severely lacking. A staged anaerobic fluidized membrane bioreactor (SAF-MBR) was used to assess the removal of four antiviral drugs, including acyclovir, abacavir, emtricitabine, and lamivudine (McCurry et al., 2014). The study found that acyclovir, abacavir, and lamivudine were efficiently removed while emtricitabine showed moderate removal when operated with a hydraulic retention time (HRT) of 6.8 h and a sludge/solid retention time (SRT) of 36 days. Although the results are promising, staged anaerobic fluidized bed membrane bioreactor (SAF-MBR) is a new technology which is not widely commercially available (Yoo et al., 2012) and may not be representative of standard anaerobic digestion. Research that has been performed examining the efficacy of anaerobic

digestion on the removal of micropollutants is inconsistent. As an illustration, the removal efficiencies of mesophilic anaerobic digestion (MAD) and thermophilic anaerobic digestion (TAD) on common micropollutants were found to be significant different for the removals of Ibuprofen (IBF), Naproxen (NPX), and Triclosan (TCS) (Gonzalez-Gil et al., 2016; Samaras et al., 2014). IBF removal efficiencies ranged from 20 – 30% to 90 – 100%, NPX ranged from 80 – 90% to 90 – 100%, and TCS ranged from 15 – 25% to 60 – 80%. Other common micropollutants such as estrone and Estradiol showed more consistency with no degradation found during the anerobic process (Congiloski et al., 2020; Gonzalez-Gil et al., 2016). The failure of consistent results makes it difficult to determine if anaerobic digestion can be an effective agent for the removal of antivirals making further research necessary before any claims can be made.

Activated sludge treatment is a common aerobic method for the treatment of municipal sewage. The main process involved in activated sludge is the ability of the aerobic micro-organisms to digest organic matter and form flocs, allowing them to settle out and be easily removed (Ranjit et al., 2021). The effectiveness of activated sludge varied greatly depending on the antiviral being examined. The degradation of the antiviral drugs abacavir, emtricitabine, and lamivudine was examined via activated sludge using both bench-scale experiments and full-scale processes (Funke et al., 2016; McCurry et al., 2014). The degradation of abacavir was studied in both a benchtop scale which found a half life ($t_{1/2}$) of 0.44 ± 0.003 h and a k_{biol} of 55.8 ± 1.8 L d^{-1} g_{ss}^{-1} and in a full-scale treatment plant which found high removal (70 – 80%) in a WWTP with an HRT of 9.9 – 11.4 h and a SRT of 24.2 – 27.7 days (Funke et al., 2016; McCurry et al., 2014). The same studies examined emtricitabine which had $t_{1/2}$ = 12.8 ± 0.07 h and a k_{biol} of 1.95 ± 0.05 L d^{-1} g_{ss}^{-1} and only minimal removal (40 – 60%) in full-scale treatment. These studies also looked at lamivudine which possessed a $t_{1/2}$ = 8.7 ± 0.06 h and a k_{biol} of 2.88 ± 0.05 L d^{-1} g_{ss}^{-1}

and high removal (70 – 80%) in full scale treatment. The research agrees that abacavir and lamivudine are removed efficiently via activated sludge, however, emtricitabine showed significantly lower removal in the full-scale process. Nevirapine and efavirenz removal in WWTPs resulted in neither drug experienced any significant change in concentration through two aeration tanks in a facility in Gauteng, South Africa (Schoeman et al., 2017). This suggests that nevirapine and efavirenz are resistant to biodegradation in the activated sludge process. A bench scale study determined the half life of acyclovir of 5.3 h and the degradation rate constant 4.9 ± 0.1 L g_{ss}^{-1} d^{-1} with complete conversion in 24 h (Prasse et al., 2011); a full-scale study found a removal efficiency of between 60 – 70% for an HRT of 9.9 – 11.4 h and a SRT of 24.2 – 27.7 days (McCurry et al., 2014). Based on this information, acyclovir is likely not completely removed in conventional activated sludge treatment. Oseltamivir carboxylate was found to have minimal removal via activated sludge process, with only 20 – 40% removal in a 30-day incubation period, this degradation however increased to >50% removal upon the addition of a *Phanerochaete chrysosporium* granular bioplastic (Accinelli et al., 2010). This suggests that oseltamivir carboxylate is not removed significantly from conventional activated sludge treatment.

2.4.3 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are other, less common methods of organic removal due to them being relatively new compared to biological and mechanical treatment methods, and often expensive. Advanced oxidation processes involve the *in-situ* generation of the hydroxyl radical, a very strong oxidant which is used to accelerate the oxidation process (Bolton et al., 2001). Many organic compounds are resistant to conventional biological degradation processes, or may be toxic to the microbial process, thus the use of AOPs to

mineralize them or convert them to biodegradable compounds is vital (Stasinakis, 2008). The use of AOPs has proven to be a strong option for the attenuation of organic micropollutants (Miklos et al., 2018). AOPs can be categorized into ozone-based, UV-based, electrochemical, and catalytic processes (Miklos et al., 2018). These processes have been briefly examined with respect to antiviral drugs, but significantly more work needs to be done to fully understand the process.

Ozone treatment is commonly used due to its high oxidizing capacity and the removal of halogenated compounds from effluent that arise from chlorine treatment. The process, however, comes at a higher cost due to the requirement to produce the ozone directly on site (Rice, 1997). Ozone, which has an oxidation potential of 2.07 V (relative to H_2 electrode) has a higher potential than other common disinfectants such as chlorine (Ikehata et al., 2005). Ozonation of the antiviral metabolite oseltamivir carboxylate was examined by a few studies; a bench scale study found an ozone degradation rate of 1.7 (±0.1) x 10^5 $M^{-1}s^{-1}$ at pH 7 with ozone doses ranging from 0.05 – 0.5 mg $O_3 \cdot mg^{-1}$ DOC (dissolved organic carbon) (Mestankova et al., 2012), and a full-scale study in Kyoto found a removal rate of >90% in ozone tertiary treatment (Ghosh et al., 2010). This data suggests that ozone is effective for the removal of oseltamivir carboxylate but fails to examine the effectiveness on the parent drug oseltamivir phosphate. Two studies examined the ozonation of the antiviral drugs abacavir, emtricitabine, and lamivudine. A pilot scale study found that ozonation was able to reduce the concentration of abacavir, emtricitabine, and lamivudine including their carboxy-transition products to below the limit of quantification when treated with an ozone dose of 0.83 ± 0.15 g $O_3 \cdot g^{-1}$ DOC and an HRT of 20 min (Funke et al., 2016). Another pilot scale study noted removal rates of 92%, 97%, and 94% for carboxy-abacavir, carboxy-emtricitabine, and carboxy-lamivudine, respectively with an ozone

consumption of 0.98 ± 0.24 g $O_3 \cdot g^{-1}$ DOC and an HRT of 17 ± 3 min (Ternes et al., 2017). These studies on abacavir, emtricitabine, and lamivudine suggest efficient removal via ozonation. A study examining the removal efficiency of amantadine by ozonation found a removal efficiency of >90% in a full-scale treatment plant in Kyoto throughout the whole process (Ghosh et al., 2010) suggesting effective removal. Several studies have examined the ozonation of acyclovir and carboxy-acyclovir; Ternes et al., (2017) found elimination of 99% for carboxy-acyclovir with an ozone consumption of 0.98 ± 0.24 g $O_3 \cdot g^{-1}$ DOC and a HRT of 17 ± 3 min; Prasse et al. (2012) found oxidation rate constants for molecular ozone to be 1.8 x 10^4 $M^{-1}s^{-1}$ for the neutral form and 3.4 x 10^6 $M^{-1}s^{-1}$ for the deprotonated form of carboxy-acyclovir using a large excess of O_3 and sampling at intervals of 10 – 15 s. These studies suggest that ozone is effective for the removal of many resilient antivirals however there is little information on the mechanism or resulting byproducts from this method, leaving room for continual research.

Photochemical advanced oxidation processes work to produce hydroxyl radicals via reaction of species such as H_2O_2 and O_3 with UV light, this process can be catalyzed to speed up the process using Fe^{2+} ions (Fenton process) or via heterogeneous catalysis using species such as TiO_2 (Litter & Quici, 2010). A pilot-scale study examining the effectiveness of tertiary treatment of micropollutants tested photocatalyzed 254 nm UV/TiO_2 processes and found oseltamivir removal of 3 ± 4%, 19 ± 1%, and 40 ± 0% with an influent concentration of 36 ng L^{-1} and oseltamivir carboxylate removal of 3%, 6%, and -2% with an influent concentration of 124 ng L^{-1} for UV dosages of 800, 2400, and 7200 $J \cdot m^{-2}$ respectively with an HRT of 18 s (Kovalova et al., 2013). Another study examining the effects of AOPs on oseltamivir carboxylate found hydroxyl radicals produced by photolysis of H_2O_2 at a bench-scale produced a rate constant of 4.7 (±0.2) x 10^9 $M^{-1}s^{-1}$ with a H_2O_2 concentration of 10 mM (Mestankova et al., 2012). There is

significant deviation in the findings of these studies, potentially due to the scale of the experiments or due to the presence of H_2O_2. A bench-scale study examining the effectiveness of photochemical AOPs for the removal of acyclovir found a rate constant of $1.23 \pm 0.07 \times 10^9$ $M^{-1}s^{-1}$ for UV_{254}/H_2O_2 with an average fluorescence rate of 4.7 mW cm^{-2}, suggesting effective removal via this process (Russo et al., 2017). Two studies examined the efficacy of photochemical degradation for the antiviral drug lamivudine; a study by Feliciano et al. (2020) found degradation up to 95.6% with 5 and 10 mg L^{-1} lamivudine solutions after 120 minutes using a UV/H_2O_2 process with 250 mg L^{-1} H_2O_2 and UV-C radiation. A study by An et al. (2011) found a degradation rate constant up to 0.0542 min^{-1} using TiO_2 catalyzed photodegradation with a 365 nm mercury lamp with a light intensity of 0.38 mW cm^{-2}. These studies suggest removal of lamivudine is possible but very slow under photochemical conditions; potential further research into this antiviral is likely necessary to improve efficiency. Amantadine was examined in two separate catalyzed photochemical studies; Zeng et al. (2015) found a removal efficiency of only 17.1% after 60 minutes via the Fenton process, and a study by An et al. (2015) using TiO_2 catalyzed photodegradation found rate constants of 0.076 min^{-1} and 0.084 min^{-1} for 1-amantadine and 2-amantadine, respectively. These studies suggest that photochemical oxidation is insufficient for the removal of amantadine. Significantly, more research needs to be placed into photochemical AOPs, as there is a lack of information on the removal mechanisms and best catalytic practices for treatment of antiviral drugs and their metabolites.

Electrochemical oxidation has been found to be an effective and environmental conscious way to mineralize non-biodegradable organic matter, allowing it to be easily removed during treatment processes (Anglada et al., 2009). The process can be categorized into two types of oxidations: direct oxidation involves the diffusion of pollutants onto the anode surface where

they are oxidized, and indirect oxidation involves the generation of a strong oxidizing agent at the surface of the anode (Garcia-Segura et al., 2018). The process has a few design considerations that need to be examined when designing the process, these include choice of an electrode material that effects selectivity and efficacy of the process, cell configuration which is needed to maintain high mass transfer rates, and operating conditions such as current density and temperature (Angalada et al., 2009). A study examining the degradation of hydroxychloroquine via electrochemical oxidation with a boron-doped diamond anode found a rate constant of 0.0226 min^{-1} for direct electrochemical oxidation, 0.0402 min^{-1} for combined sonication, and 0.0842 min^{-1} for combined UV radiation with HCQ concentration of 250 mg L^{-1} and current intensity of 20 mA cm^{-2} (Bensalah et al., 2020). This data showed the complete removal of hydroxychloroquine from aqueous solution suggesting strong a capacity for degradation. Zhou et al. (2019) examined the removal of abacavir using a porous Ti/SnO_2-Sb tipped anode where a degradation efficiency of 97% was found in 10 minutes with a current density of 0.2 mA cm^{-2} and a corresponding rate constant of 0.36 min^{-1}. This study proposes high efficiency for the removal of abacavir but also states a low TOC removal of 3.7% in 10 minutes, suggesting the possibility of high selectivity in the process. The removal of lamivudine was examined using a Ti/ SnO_2-Sb/Ce-PbO_2 anode electrochemical process using bicarbonate as an enhancement was able to achieve degradation rate constants ranging from 0.129 – 0.144 min^{-1} and corresponding removal efficiencies from 91.4 – 96.0% (Wang et al., 2019). The same study found increasing HCO_3^- resulted in increasing rate constant up to 14.53 min^{-1} and decreasing energy consumption from 28.97 to 0.58 Wh L^{-1} at 50 mM, which they attribute to the formation of $CO_3^{\cdot -}$. Electrochemical AOPs are promising technologies that, with further research into methods and catalysts, may prove to be effective methods for treatment of resilient antivirals.

2.5 Environmental Fate of Antiviral Drugs

Antiviral drugs, like other pharmaceuticals, could enter water systems due to incomplete metabolization in patients and further urinary excretion (Prasse et al., 2010). Wastewater treatment plants are unable to remove all these drugs from the wastewater, resulting in significant quantities being discharged from the facilities (Prasse et al., 2010). An overview of how antiviral drugs enter ground and surface waters is shown in Figure 2.3. There is a lack of information regarding the specific modes of transport in the natural environment, however, data from other drugs suggest that the hydrogeological conditions, dissolved organic matter, and physical conditions of the aquatic environment may affect the movement of antivirals (Kumar et al., 2020). The concentrations of many antiviral drugs have been documented in natural waters such as rivers, lakes, and groundwaters and have been proven to be persistent in some cases as seen in Table 2.4 (Jain et al., 2013). Factors affecting the persistence of antiviral drugs include photodegradation, adsorption, and biodegradation.

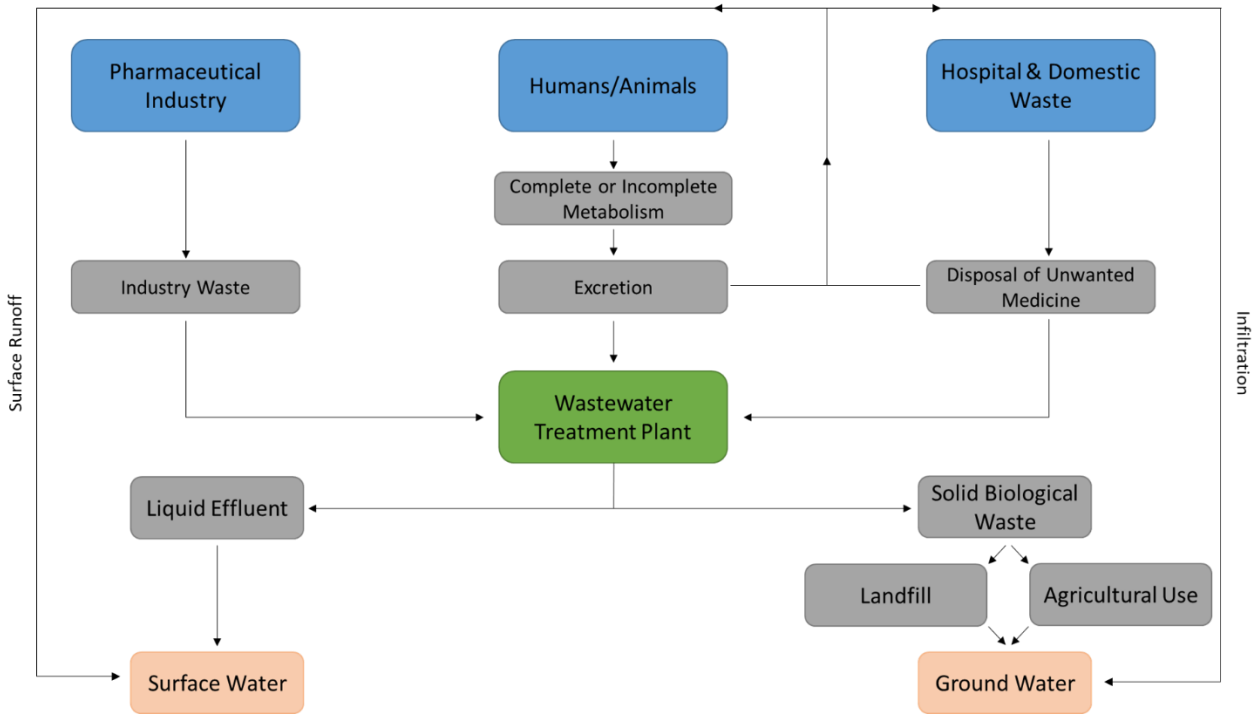


Figure 2. 3 Pathway of Antivirals into Aquatic Environment from Various Sources.

Table 2. 4 Concentrations of Antiviral Drugs Found in Natural Water Systems

Antiviral Drug	Highest Reported Concentration	Location	Reference
Oseltamivir	165.9 ng L^{-1}	Japan	Takanami et al., 2012
Oseltamivir Carboxylate	556.9 ng L^{-1}	Japan	Takanami et al., 2012
	58 µg L^{-1}	Japan	Söderström et al., 2009
Abacavir	< 43.1 ng L^{-1}	South Africa	Wood et al., 2015
	92 ng L^{-1}	Germany	Funke et al., 2016
	220 ng L^{-1}	Germany	Prasse et al., 2010
emtricitabine	< 33 ng L^{-1}	South Africa	Mlunguza et al., 2020
	280 ng L^{-1}	Germany	Funke et al., 2016
Efavirenz	< 519.0 ng L^{-1}	South Africa	Wood et al., 2015
	< 380 ng L^{-1}	South Africa	Mlunguza et al., 2020
	148 ng L^{-1}	South Africa	Wooding et al., 2017
Nevirapine	1480 ng L^{-1}	South Africa	Wood et al., 2015
	227 ng L^{-1}	South Africa	Wooding et al., 2017
	< 410 ng L^{-1}	South Africa	Ngumba et al., 2020
	6 µg L^{-1}	Kenya	K'oreje et al., 2016
Ritonavir	< 156.6 ng L^{-1}	South Africa	Wood et al., 2015
	489 ng L^{-1}	South Africa	Wood et al., 2017
Acyclovir	750 ng L^{-1}	Germany	Funke et al., 2016
	1800 ng L^{-1}	Germany	Prasse et al., 2010
Lamivudine	230 ng L^{-1}	Germany	Funke et al., 2016
	720 ng L^{-1}	Germany	Prasse et al., 2010
	167 µg L^{-1}	Kenya	K'oreje et al., 2016

Photolysis is a process which involves the absorption of light energy to promote an electron into an excited state; this excited state allows the molecule to undergo chemical reactions which cannot take place in ground state conditions (Jordaan and Shapi, 2017). The photolysis transformation pathway is important for many organic pollutants as it predicts the outcome of these molecules in surface waters (Zhou et al., 2015). Direct photolysis of antivirals

is generally ineffective. Studies have shown that the drugs remdesivir, nevirapine, lamivudine, acyclovir, oseltamivir, oseltamivir carboxylate, abacavir, emtricitabine, ritonavir, and amantadine are resistant to direct photolysis (Avataneo et al., 2020; Bhembe et al., 2020; Zhou et al., 2015; Bartels and von Tümpling, 2008; Vukkum et al., 2012; Hamarapurkar and Parate, 2013; Nageswara Rao et al., 2010; Azuma et al., 2017). Indirect photolysis is transformation due to reaction with intermediates formed via photolysis of other compounds, has been shown to be more effective against acyclovir, lamivudine, and oseltamivir carboxylate (Bartels and von Tümpling, 2008; Zhou et al., 2015). Studies have suggested that direct photolysis is however effective for the transformation of hydroxychloroquine and favipiravir, suggesting their environmental persistence is limited (Azuma et al., 2017; Dabić et al., 2019). Many studies have examined photodegradation of antivirals, however, few of them examined these processes in real water matrices, leaving the environmental fate of many compounds' unknown.

Adsorption is another pathway in which antiviral drugs move in a natural water system, this pathway involves the antiviral drugs adhering onto sediments in the water, losing mobility. Natural adsorption is not a method of removal or degradation of antivirals but a change in mobility. Non-polar organic micropollutants such as some antivirals which adsorb to sediments have low bioavailability to marine animals, but also result in bioaccumulation (Neff et al., 1984). This bioaccumulation may result in a less wide-spread issue but may cause more significant negative effects in certain locations. Antiviral drugs which have a higher value of solubility directly correlate to a higher level of bioavailability (Neff et al., 1984); higher solubility correlates with a greater range of potential contamination as well as exposure to a greater variety of species. Little information is available regarding the natural sorption of antivirals in natural waters, and thus it is unclear how many of these pollutants behave. A study of Sorption of

antiviral drugs found that coefficients of sorption to river sediment (log K_d) values fell in the range of 0.1 – 0.14, which is approximately 3 – 4 orders of magnitude less than those reported by many other pharmaceuticals (Azuma et al., 2017). Among the drugs tested were amantadine, favipiravir, oseltamivir, and oseltamivir carboxylate; the study suggests that the extent of adsorption onto river sediments for these antivirals is negligibly small.

Biodegradation of antivirals in the environment is a method of determining how long these drugs will remain in their current form. Microorganisms present in natural waters have the potential to breakdown organic materials and use them as an energy source, however some organic compounds are more susceptible to this process than others. Research on the natural biodegradation of antivirals is very limited, however it is believed that the majority of antiviral drugs are resistant to these processes. A study by Azuma et al. (2017) determined that the biodegradability of oseltamivir was high, but biodegradability of amantadine, favipiravir, and oseltamivir carboxylate were very limited in river water. A study found that oseltamivir carboxylate had a half life of around 18 days but failed to determine if the main factor was biodegradation or indirect photolysis (Bartels and von Tümpling, 2008). Research by Vanková (2010) determined the percent biodegradability for nevirapine and lamivudine to be 3% and -3% respectively. These studies reinforce the assumption that natural biodegradation of antivirals is very limited.

The fate of antivirals in natural waters is lacking information, and this lack of research is limiting our ability to fully understand the scale of the problem. Currently, there is significantly more research looking into preventing the release of these pollutants, but more focus needs to be made to determine the repercussions of treatment. It is vital to understand the the potential hazardous effects of antiviral drugs in the environment to provide information on which drugs

are the highest concern. An understanding of which drugs are present in local wastewaters and which drugs cause the most potential harm will allow researchers and plant operators to focus their attention on the most pressing issues.

2.6 Environmental Toxicological Effects of Antivirals

Antiviral drugs create a serious potential issue due to their toxicological effects in the environment. Environmental issues related to the toxicology of antivirals are complex and may be present in different ways. Effects that these drugs may have on the environment include drinking water contamination, toxic effects towards marine organisms, and development of resistance (Schwarzenbach et al., 2006). The antiviral drugs themselves are not the only cause of toxicological concern, studies have suggested that the metabolites, photoproducts, and oxidation products may be equally so or more toxic than the parent compounds (Prasse et al., 2012; Dabic et al., 2019). Therefore, it is vital to have a complete understanding of the potential effects of these drugs so that policy- and lawmakers can make informed decisions regarding which pollutants must be treated and removed.

Environmental toxicological effects are vitally important to understand, exposure to these drugs can cause issues such as genotoxicity and endocrine disruption, making it a significant environmental problem (Feliciano et al., 2020). The ecotoxicity of antiviral drugs is poorly understood and under-studied, with only a small portion of them having any relevant information. One study examining the environmental effect of triazoles found Ribavirin had no significant toxicological effect on zebrafish embryo or *Daphnia Magna*, suggesting that its potential impact is relatively low (Durjava et al., 2013). The toxicological effects of oseltamivir were studied on both fish and daphnia, both of which did not show any effect at concentrations as high as 1 mg L^{-1}, suggesting low ecotoxic potential (Straub, 2009). Efavirenz is expected to be

environmentally hazardous due to its environmental persistence, a study found that dosing the fish *O. Mossambicus* with as low as 10.3 ng/L efavirenz may lead to increased risk of developing liver damage, functional organ loss, and declines in fish health (Robson et al., 2017). Nevirapine has shown mixed results in toxicological testing. Oliveira et al. (2014) found that nevirapine dosing did not lead to any acute toxicity or DNA damage in mice; however, Wood et al. (2016) found an average Inhibitory concentration-50 (IC_{50}) of 0.03 ug/L for 293T cells, suggesting significant toxic effects.

The toxicological impact of some antivirals does not come from the parent drug, but instead from the degradation byproducts produced during treatment. In the case of lamivudine, a study examined the toxicity of pre- and post-treatment lamivudine solution on L. *Sativa* which found an average seed germination decrease from 8.33 for control, to 5 for untreated solution, to 3 for photo-Fenton treated solution, suggesting that toxicity of lamivudine increases as byproducts are formed (Feliciano et al., 2020). A drug of significant interest recently is acyclovir, which has been shown to have a particularly toxic disinfection byproduct N-(4-carbamoyl-2-imino-5-oxoimidazolidin) – formamido-N-methoxyacetic acid (COFA). Acyclovir was shown to be non-toxic to both D. rerio and D. Magna suggesting that the drug has low toxicity (Schutler-Vorberg et al., 2015). Carboxy-acyclovir, the metabolite of acyclovir, was found a significant increase in toxicity towards D. magna (Schutler-Vorberg et al., 2015) but no bacterial toxicity was observed for V. *fischeri* (Prasse et al., 2012). COFA however was found to be toxic toward algae and V. *fischeri*, significantly decreasing growth rate in both species (Schutler-Vorberg et al., 2015; Prasse et al., 2012).

Development of drug resistance in environmental communities is especially under-researched for antiviral drugs, and very little is known about the mechanisms or possibility of

resistance development. A study found that treating mallard ducks infected with influenza A and H1N1 with oseltamivir caused the mutation H274Y in the neuraminidase gene, causing resistance to the drug (Jarhult et al., 2011). This development was found by exposing the ducks to just 1 μg L^{-1} oseltamivir carboxylate for 5 days in their water, suggesting that even very low oseltamivir concentrations can cause significant effects

Toxicology of antivirals requires significant further research, as many antivirals have not seen any data towards their potential toxicological effects. Testing more drugs, their byproducts, and testing against a greater variety of species would be a strong first step in improving out knowledge of environmental toxicology.

2.7 Future Research Directions

Information in this literature review discussing the types, occurrence, detection methods, treatment methods, environmental fate, and toxicological effects of antiviral drugs, shows that there are gaps in our current knowledge. This paper describes the lack of removal of many antiviral drugs such as oseltamivir, emtricitabine, efavirenz, nevirapine, atazanavir, ritonavir, and amantadine by conventional wastewater treatment systems such as mechanical and biological treatments. Advanced oxidation processes such as ozone treatment and UV/H_2O_2 processes have been shown to improve degradation for antiviral drugs including ritonavir, oseltamivir, and emtricitabine. The analytical methods as well as their limits of detection in various water matrices are also shown in the paper. It was found that HPLC-MS used with an SPE sample preparation is the most common way for quantification of antiviral drugs. The antiviral drugs efavirenz and nevirapine were found to have potentially toxic environmental effects while lamivudine and acyclovir were found to have toxic disinfection byproducts.

Biological treatment is a very important aspect of treatment plants worldwide, however many studies have shown that it is ineffective for the removal of antiviral drugs. Anaerobic digestion has only been lightly researched on its ability to remove antiviral drugs, and within this research there has been significant variability between studies. Further research needs to be done both for examining a greater variety of antiviral drugs and more studies attempting to replicate previous results to ensure the results being produced are accurate. Aerobic biological treatments have had significantly more research done however information is still lacking for many antiviral drugs and often the kinetics or mechanisms of removal are not discussed in detail. Further research needs to be done in bench scale studies examining mechanisms, kinetics, and factors affecting each to establish a better overall understanding of the overall process.

Advanced oxidation processes such as ozone, photochemical, and electrochemical disinfection have shown a lot of promise towards the removal of antiviral drugs however there are many information gaps that still need to be filled. For many antivirals, there has been little to no information regarding how effective the removal is, and even fewer studies discussing the reaction mechanisms, leaving many unanswered questions about how effective these processes really are. Another issue surrounding advanced oxidation processes is the shortage of studies examining full scale systems, determining if the processes are as effective in full- or pilot-scale as they are in bench-scale experiments is vital to introducing the new processes on a global scale. Ozonation has been shown to be effective for the removal of many resilient antivirals, and it has been argued that it has a lower risk of producing toxic disinfection byproducts due to halogens not being introduced, however it has been shown that even ozone disinfection can lead to production of toxic products (Prasse et al., 2012). Further studies need to be done examining the toxicity of antiviral solutions before and after disinfection, this will help to identify possible

issues stemming from these treatment methods. Photocatalytic processes have been shown to improve degradation efficiency compared to traditional photochemical degradation, suggesting that further research into different methods or materials for catalysis may prove to be very useful when looking at especially resilient antivirals.

One significant area of research that is currently lacking is the knowledge of byproducts, intermediates, or disinfection products. Knowledge of these potential pollutants being created from treatment is vital, as it has been reported in the literature they can be just as or more dangerous as the parent compounds. Increased knowledge in this area first needs to come from monitoring pathways of degradation from multiple processes, and the quantity of these species being formed. Further research needs to be conducted to determine the toxicity and environmental fate of treatment byproducts, this will help us determine which antiviral drugs and which treatment methods generate the largest risk of dangerous byproduct production. Increased knowledge around these byproducts will also create the requirement for further research to be conducted on the capability of various treatment options to remove these byproducts, and methods for detection of the potentially huge number of products being produced in both wastewater and natural water. Disinfection byproducts of antivirals seems to be an area that has been relatively ignored by much of the scientific community, with few articles focussing on the potential damage these products may have.

The environmental fate of antivirals is an area which has seen minimal research which could prove detrimental to some ecosystems. Information on where antiviral drugs accumulate, depending on their adsorption, infiltration, or solubility, is relatively unknown. Further information on how antiviral drugs act in their environment is important to predict whether drugs may end up accumulating in lakes, groundwaters, or adsorbed on to sediments. The time for

which antivirals persist in the environment is also lacking information, drugs that tend to be persistent may have a higher prevalence in biological communities increasing the potential for development of resistance and toxic effects. Finally, the toxic effects of both the antiviral drugs and their byproducts needs to be examined to a greater extent. Studies must focus on greater varieties of compounds and on more diverse species, every species will react differently to exposure, thus it is unrealistic to assume that a lack of toxicity in one species will prove to be equivalent in others.

2.8 Conclusion

Given the lack of information on the use and consequences of advanced oxidation processes for the removal of antiviral drugs, it's clear that there is a current need for further research into this area. Complete removal of antiviral drugs from wastewater treatment plants should be a necessity for modern facilities. Furthermore, understanding the consequences of our treatment including how non-degraded substances impact local ecosystems and the byproducts that are produced during treatment need to be better understood. Among the possibilities for antiviral removal, ozonation and electrochemical processes appear to be promising effective methods for especially difficult pollutants. Research into optimization of conditions will be required to achieve the most efficient treatment of antiviral drugs in order to provide the highest possible quality effluent.

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Chapter 3: Removal of the Antiviral Drug Oseltamivir by Ozonation and Analysis of Contributing Factors

3.1 Introduction

Nearly a third of people on Earth do not have access to safely managed drinking water and only two fifths have access to safely managed sanitation services, resulting in billions of people who are left without their right to enjoy clean water, sanitation, and other relating benefits (WWAP, 2019). The lack of clean water access is being exaggerated by drastic population growth causing greater demand and thus greater competition in densely populated areas (Teklehaimanot et al., 2015; Vatankhah et al., 2019). The long-term effects of chemical pollution are largely unknown with respect to aquatic life or human health (Schwarzenbach et al., 2006). It should therefore come as no surprise that properly managed water treatment facilities are vital for successful society.

Micropollutants are compounds which are found in the μg L^{-1} or ng L^{-1} concentration range in water, soil, and wastewater; over time they have become a growing concern in the scientific community due to steadily increasing concentrations (Virkutyte et al., 2010). Types of micropollutants entering aquatic environments include pharmaceuticals, personal care products, steroids, hormones, industrial chemicals, and pesticides (Luo et al., 2014). Wastewater treatment plants are also not specifically designed to remove micropollutants, allowing them to pass through treatment processes unchanged (Luo et al., 2014). Antiviral drugs are a subclass within the micropollutants category that are used specifically for treating viral infections such as HIV, herpes virus, hepatitis B and C, and influenza (He, 2013)

Oseltamivir or Tamiflu® (ethyl (3R,4R,5S)-5-amino-4-acetamido-3-(pentan-3-yloxy)cyclohex-1-ene-1-carboxylate) is a neuraminidase inhibitor which works to prevent replication of influenza A and B viruses (McCellan, 2001). The persistence of oseltamivir in the environment is not well known, studies suggest that oseltamivir shows relatively high persistence towards biodegradation (Accinelli et al., 2010; Prasse et al., 2010); however, others suggest that direct monochromatic UV-C irradiation can remove OSP (oseltamivir phosphate) (Tong et al., 2011). The photodegradation of oseltamivir is relatively slow and it is suggested that the resulting transformation products are more persistent than the parent compounds (Goncalves et al., 2011).

Studies have detected the presence of oseltamivir and transition products in natural waters at relatively high levels. The presence of oseltamivir and oseltamivir carboxylate (OC) has been monitored at WWTP locations with concentrations ranging from 16 – 482 ng L^{-1} for OC and 7 – 159 ng L^{-1} for oseltamivir phosphate (OSP) (Ghosh et al., 2010; Prasse et al., 2010; Simazaki et al., 2015; Takanami et al., 2012). River water sampling has also detected the

presence of oseltamivir at elevated levels in Spain and Japan, with concentrations ranging from 50 – 482 ng L^{-1} for OC and 100 – 159 ng L^{-1} for OSP (Goncalves et al., 2011; Soderstrom et al., 2009; Takanami et al., 2012). Elevated levels in riverways may be due to the phenomenon reported in multiple papers suggesting the accumulation of OSP through WWTP processes (Prasse et al., 2010; Kovalova et al., 2012).

The effects of oseltamivir release in the environment are largely unknown, however studies already suggest there may be serious outcomes related to OSP release in environmentally relevant concentrations. Pandemic scenarios result in increased usage of antiviral drugs such as oseltamivir which may lead to increased concentration in natural waters (Singer et al., 2007). Some suggest that this high concentration may lead to the development of resistant viral genes due to waterfowl coming in close contact (Singer et al., 2007) while others state that there is no significant risk in either surface waters or sewage works (Straub, 2009). Studies suggest that exposure to OSP and OC results in a neuraminidase I222T substitution in all viruses sampled from oseltamivir-introduced mallard ducks, and that this resistant variant has the potential to circulate within various wild birds (Gillman, 2016; Gillman et al., 2015; Järhult, 2012). It is suggested that this amino acid substitution leads to changed antiviral sensitivity in an influenza A subtype that can be highly pathogenic in humans (Gillman et al., 2016).

3.2 Materials & Methods

3.2.1 Chemicals and Reagents

Oseltamivir phosphate (OSP) (MW = 410.4 g mol^{-1}) pharmaceutical secondary standard was supplied by Sigma-Aldrich, Canada. A stock solution for OSP was prepared at 50 µM using ozone demand-free water (ODFW) as the solvent kept in a borosilicate dark glass bottle and stored at 4 ^{o}C in the dark. Other chemicals utilized were sodium chloride, potassium chloride,

sodium nitrate, sodium sulfate anhydrous, calcium chloride dihydrate, magnesium chloride hexahydrate, sodium hydroxide, potassium phosphate dibasic anhydrous, potassium phosphate monobasic, sodium phosphate monobasic anhydrous, and o-phosphoric acid that were obtained from Fisher Scientific Canada. Sodium bromide, sodium thiosulfate, and potassium indigo trisulfonate were supplied by Sigma-Aldrich Canada; tert-butyl alcohol supplied by Acros Organics, and ammonium chloride supplied by Caledon Laboratories Canada. ODFW was prepared by bubbling ozone through MilliQ water for 60 minutes followed by allowing minimum 2 days to allow gassing off any residual ozone. Phosphate buffer was prepared in a stock solution at a concentration of 50 mM with a mixture of mono- and di-basic potassium phosphate to produce a neutral pH 7 buffer and stored at 4 ^{o}C in the dark. Sodium thiosulfate quencher at a concentration of 50 mM was prepared in ODFW and stored in the dark at 4 ^{o}C until used. Glassware was stored in ODFW to minimize any ozone-demand introduced into experiments.

3.2.2 Experimental Setup

3.2.2.1 Photodegradation Experiments

Photodegradation of Oseltamivir was conducted using a 1000 W medium pressure collimated UV lamp with an irradiance of 2.62 mW cm^{-2}. Solutions were irradiated in circular 80 mL reaction vessels with magnetic stirrers used to agitate the sample. Samples were exposed for 5 minutes under direct radiation with aliquots taken at pre-determined time intervals and kept in the dark at 4 ^{o}C until analysis. Tests were carried out in MilliQ water with 0.01 mM OSP and 0.02 mM pH 7 phosphate buffer. The absorbance spectrum of 0.01 mM OSP solution in MilliQ water was obtained using an Ultraspec 2100 Pro UV/Visible Spectrophotometer.

3.2.2.2 Ozonation Experiments

Ozonation experiments were carried out in 20 mL brown borosilicate glass vials with airtight septum caps. Prior to the addition of O_3 the required volumes of ODFW, buffer, OSP stock solution, and matrix solution were added to the reaction vials. Ozone stock solution was prepared, and the concentration was determined via the indigo method described in the article by Bader (1981). The ozone stock solution was kept covered in an ice water bath to ensure minimal concentration losses during experimental procedures. The required volume of ozone stock solution was added to the reaction vial via air-tight syringe through the septum cap, the moment when all the ozone left the syringe was considered time-zero. Samples were allowed to stir for the pre-determined time period on a multi-position magnetic stirrer before the addition of $Na_2S_2O_3$ quencher via air-tight syringe. Vials were left to stir for an additional 60 seconds after the addition of quencher to ensure complete mixing of the quencher. Samples were transferred into dark glass HPLC vials for analysis. Temperature and pH measurements of samples were taken at the beginning and end of each experiment to ensure no significant changes occurred and provide averaged values.

The second order rate constant was determined using two different methods to provide reinforcement of data. The first method used is known as the Ct method and is described in the study by Broséus et al. (2009). This method involves monitoring the concentration of both the target compound and the ozone residual over time. The second method of determination for the second order rate constant involves the determination of pseudo-first order rate constants at multiple ozone to OSP ratios while keeping one species in excess. The study by Benitez et al. (2000) describes this method in detail.

The concentrations of ions for matrix effects were chosen based on both data from local, publicly available treatment plant reports as well as scientific articles. Chloride concentration was chosen to be 250 mg L^{-1} and nitrate concentration of 0.5 mg-N L^{-1} based on the 2019 Edmonton Wastewater Annual Performance Report. Bromide concentration was set to 2 mg L^{-1} based off the ranges reported in studies in China and Switzerland (Soltermann et al., 2016; Wu et al., 2010). The concentration of sulfate used in our study was chosen to be 100 mg L^{-1} based off a study by Zhang et al. (2013) from The United States.

The O_3:DOC ratios for secondary effluent experiments were chosen based off the study by Antoniou et al. (2013) stating that active pharmaceutical ingredients that are easily degradable if $DDO_3/DOC \leq 0.7$ (DDO_3 is the ozone dose required to achieve a 1 log reduction in concentration). Oseltamivir is believed to be easily degradable based on the rate constant found in section 3.3.2. Prior to experimentation, the secondary effluent used was subjected to ion chromatography via a Dionex ICS-5000 Ion Chromatography analyzed via a TOC-L Shimadza Total Organic Carbon Analyzer to determine the DOC. Samples were then analyzed for O_3 concentration via Indigo Method, DOC via total organic carbon analyzer, and OSP via UPLC-TOF-MS.

3.2.2.3 Toxicity Experiments

The acute toxicity tests of both ozonated and non-ozonated samples of OSP in ODFW and secondary effluent were assessed via Microtox® assays with the bacterial reagent *Vibrio fischeri.* The bioluminescence inhibition screening tests were performed in triplicate in 96-well plates where osmotic adjustment solution was added to adjust the salinity to an adequate level. In another 96-well plate bacterial solutions were transferred into each well and bioluminescence was measured using a Synergy Microplate reader. Samples were then added to the 96-well plate

containing bacteria and incubated at 15 ºC for 5 minutes before the luminescence was measured again and light inhibition was calculated.

The genotoxicity of OSP samples was tested via the SOS-ChromoTest which is a colorimetric method used to determine the relative genotoxic potential of a compound. The method measures the expression of genes caused by genotoxic compounds in *Escherichia coli* via fusion with the structural gene for ß-galactosidase (Quillardet et al., 1993). In 96-well plates, triplicate samples were prepared for each sample and *E. Coli* was added to each of the well plates followed by an incubation period of 2 hours at 37 ºC. The absorbance of the samples was measured at 420 nm and 600 nm using a Synergy Microplate Reader. Following the initial incubation, blue Chromogen and alkaline phosphatase mixture was added to each well followed by another 90-minute incubation at 37 ºC. The cytotoxic effect of the samples was calculated via equation 1 using the bacterial survival rate. The genotoxicity was calculated through the growth factor (G) in Equation 2, the ß-galactosidase activity in ß-gal in Equation 3, and the induction factor (IF) in equation 4 (SOS-ChromoTest, 2019).

$$\%\ Survival\ Rate = 100 \times \frac{OD_{420}}{OD_{420\,c}} \qquad \text{Equation 1}$$

$$G = \frac{A_{420} - A_{420\,b}}{A_{420\,c} - A_{420\,b}} \qquad \text{Equation 2}$$

$$\beta - gal = \frac{A_{600} - A_{600\,b}}{A_{600\,c} - A_{600\,b}} \qquad \text{Equation 3}$$

$$IF = \frac{\beta - gal}{G} \qquad \text{Equation 4}$$

Where: OD_{420} represents the optical density at 420 nm of the sample and $OD_{420\,c}$ represents the optical density at 420 nm of the control; A_{420}, $A_{420\,b}$, and $A_{420\,c}$ represent the absorbance at 420

nm for the sample, blank, and control, respectively; A_{600}, $A_{600\ b}$, and $A_{600\ c}$ represent the absorbance at 600 nm for the sample, blank, and control, respectively.

3.2.3 Analytical Method

OSP samples were analyzed using ultra-performance liquid chromatography – quadrupole time-of-flight mass spectrometry (Xevo G2-S, Waters), operated in positive mode. Chromatographic separation was achieved using ACQUITY UPLC BEH C18, 50×2.1 mm column, at 40 °C with an injection volume of 10 µL. The mobile phase consisted of water with 0.1 % formic acid (solvent A) and acetonitrile with 0.1 % formic acid (solvent B).

3.3 Results & Discussion

3.3.1 Photodegradation

The photodegradation of OSP was examined using 1000 W medium pressure colllimated UV lamps to determine potential methods of removal for wastewater treatment systems as well as potential environmental removal methods. Based on the plot shown in Figure 3.1, the removal of oseltamivir follows a first order kinetic regime, with relatively slow removal rates. The first order photodegradation rate constant equates to 1.81×10^{-3} ($\pm 0.06 \times 10^{-3}$) s^{-1} and the fluence-based rate constant was 6.93×10^{-4} ($\pm 0.25 \times 10^{-4}$) $cm^2\ mJ^{-1}$ with a removal of just 53% ± 9% (n=2). The values reported here are similar to those reported by Goncalves et al. (2011) who examined the removal of oseltamivir ester in DI water using a solar simulator transmitting wavelengths below 290 nm. The study found a rate constant of $4.53 – 4.75 \times 10^{-6}$ s^{-1} first order rate constant which is lower than this research likely due to the lower irradiance of 0.5 mW cm^{-2} compared to the 2.62 mW cm^{-2} from our experimentation as well as the use of a wider spectrum of wavelengths.

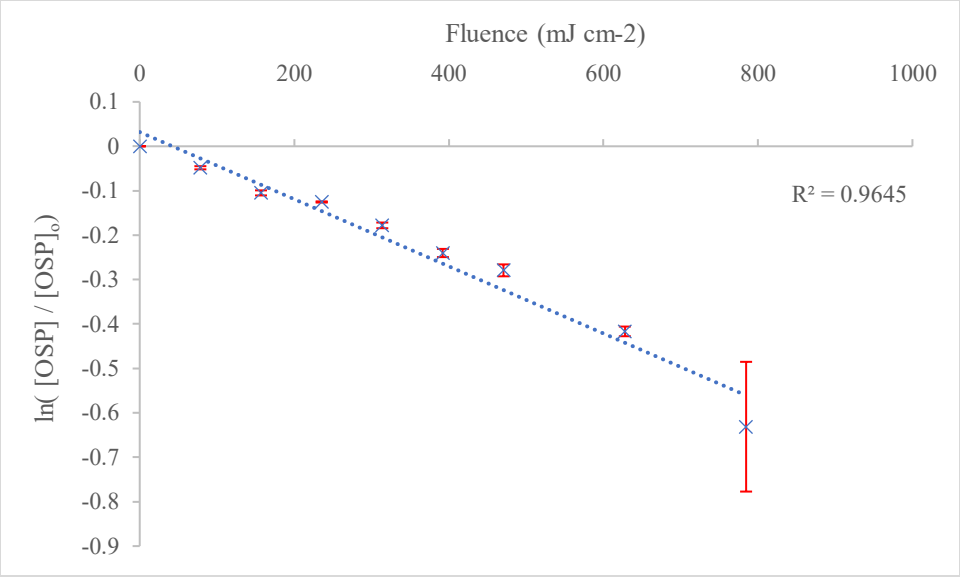

Figure 3. 1 Pseudo-first order photodegradation of OSP via collimated medium pressure radiation as a function of UV fluence (Source: 100W medium pressure columnated UV lamp, pH = 7.50, Irradiance = 3.41 mW cm^{-2}, OSP concentration = 4.1 mg L^{-1}, PO_4 buffer concentration = 3.0 mg L^{-1}).

From the rate constant, we can calculate the half-life ($t_{1/2}$) in seconds of OSP in collimated UV radiation exposure using the following Equation 5 where k is the first order rate constant in s^{-1}. The half-life calculated here is found to be 383 ± 13 seconds suggesting a long residence time is required to efficiently remove OSP in medium pressure UV treatment methods. Therefore, it is unlikely that UV treatment alone is a viable option for removal of OSP from wastewater systems, as well as unlikely that OSP is removed via direct sunlight once entered into natural water systems (Bartels and von Tümpling, 2008).

$$t_{1/2} = \frac{0.693}{k} \qquad \text{Equation 5}$$

3.3.2 Ozone Kinetics

3.3.2.1 Overall Rate Constant Determination

Ozone degradation follows a pseudo-first order rate constant when ozone is applied to the reaction in excess. The overall second order rate constant was determined using two different methods. The first method of determining the second order rate constant was via curve fitting of pseudo-first order rate constants at the five O_3:OSP ratios; as shown in Figure 3.2, the rate constant was found to be 6.79×10^3 M^{-1} s^{-1} from the slope shown in Figure A.3. The Ct method was used at two differing OSP:O_3 ratios; the 5:1 OSP ratio resulted in a second order rate constant of 8.97×10^3 M^{-1} s^{-1} and the 10:1 OSP ratio resulted in 9.10×10^3 M^{-1} s^{-1} as shown in Figure 3.3. The rate constants found here are within a similar range (10^3 – 10^5 M^{-1} s^{-1}) of other olefins with ozone, which is predicted to be the primary site of attack for oseltamivir (Dowideit and Von Sonntag, 1998). The rate constants are also discussed by Mestankova et al. (2012) who examined the degradation rate constant of oseltamivir carboxylate with both molecular ozone and hydroxyl radicals who discovered rate constants of 1.7 (± 0.1) × 10^5 M^{-1} s^{-1} with molecular ozone and 4.7 (± 0.2) × 10^9 M^{-1} s^{-1} with hydroxyl radicals at a pH of 7 - 8. The rate constants discussed by in the Mestankova study most likely differ from the values reported here due to the different rate constants with oseltamivir carboxylate versus oseltamivir phosphate.

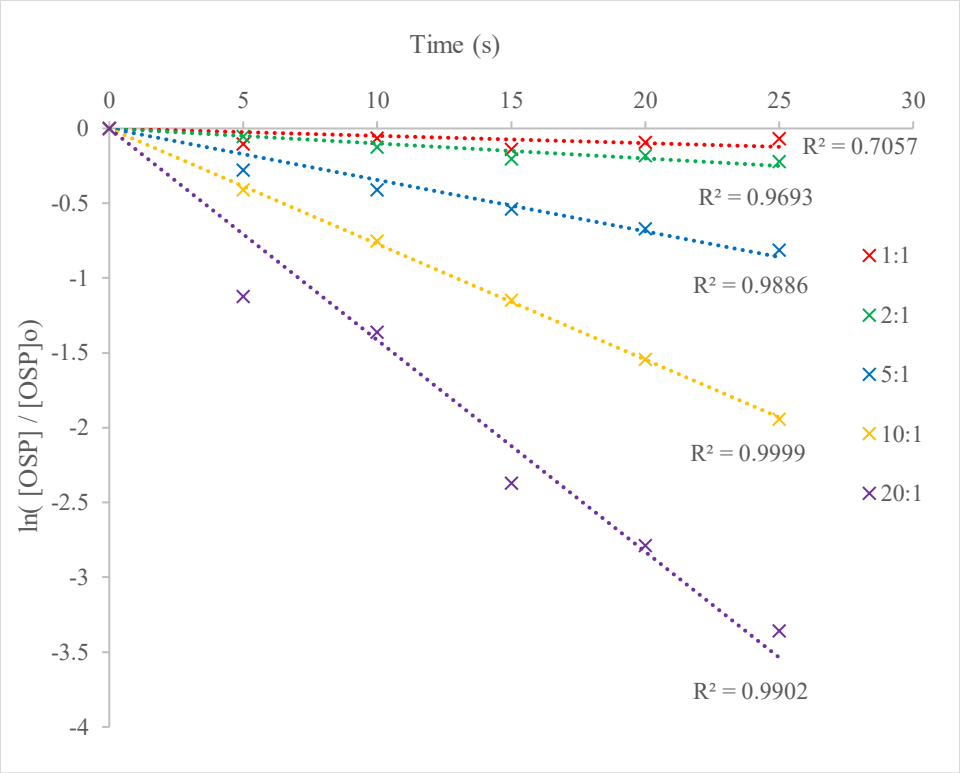

Figure 3. 2 Degradation of OSP via O_3 batch treatment at varying O_3:OSP ratios (OSP = 1.0 µM, O_3 = 1.0 - 20.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.015 – 0.30 mM, pH = 6.9 - 7.1, Temp. = 20.9 – 21.9°C).

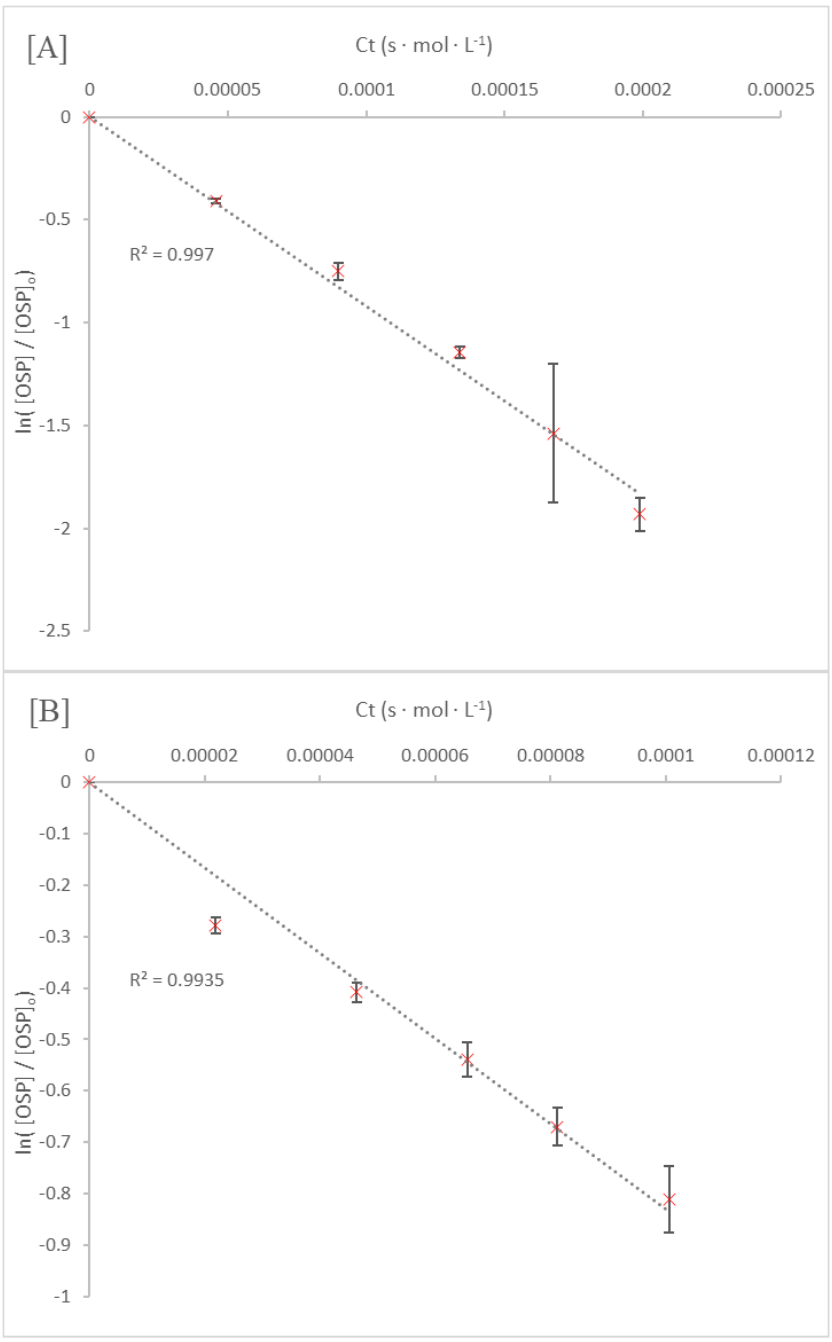

Figure 3. 3 Ct method of second-order rate constant determination for [A] 10:1 O_3:OSP molar ratio and [B] 5:1 O_3:OSP molar ratio ([A] OSP = 1.0 µM, O_3 = 10.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.15 mM, pH = 7.1, Temp. = 21.1 – 21.4ºC; [B] OSP = 1.0 µM, O_3 = 5.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.075 mM, pH = 7.1, Temp. = 21.5 - 21.7ºC).

3.3.2.2 Effect of pH on Oseltamivir Degradation

The degradation of OSP via O_3 was monitored at pH levels of 4.0, 5.5, 7.0, 8.5, 10.0, and 11.0 in order to provide a better description of the mechanism and factors that greatly impact the reaction. The results shown in Figure 3.4 show that the first-order rate constant changes very little between pH 4.0 – 7.0 and increases between pH 7.0 – 8.5. Similar results were shown in the study by Mestankova et al. (2012) with a significant increase in apparent rate constant from 2.5×10^4 to 1.7×10^5 when comparing the value at pH 3 and pH 7 – 8, respectively. Studies also showed significant increases in observed rate constant for the organic acids cinnamic acid and para-chlorobenzoic acid which both share carboxylate functional groups in close parameter with an alkene bond (Park et al., 2004; Letizke et al., 2001). This increase in rate constant may be due to the increase in hydroxyl radicals that results from increased ozone decomposition and longer hydroxyl radical lifetimes at elevated pH levels. In some cases where there is an absence of ozone fast-reacting compounds, hydroxyl radicals may result in an increase in reaction kinetics (Beltran & Fernando, 2003). At pH 8.5, the combined degradation effects of molecular ozone as well as the increased impact from hydroxyl radicals causes the larger degradation rate constant.

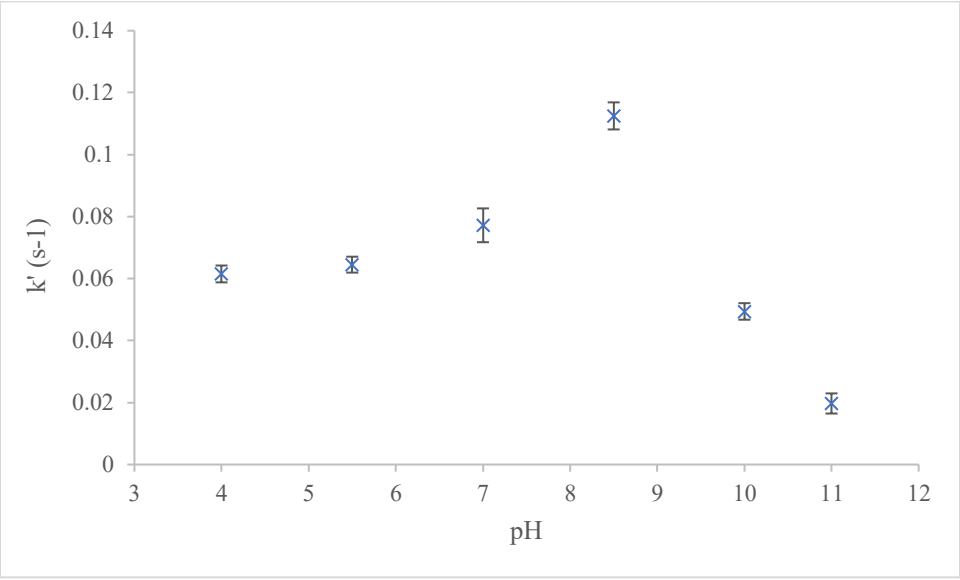

Figure 3. 4 Effect of pH on the pseudo-first order degradation of OSP via O_3 batch treatment (OSP = 1.0 µM, O_3 = 10.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.15 mM, Temp. = 21.8 – 22.6°C, NaOH & H_2SO_4 as pH adjustment).

At pH levels 10.0 and 11.0, an overall decrease in apparent rate constant is observed the OSP concentration rapidly decreases during the first 5 second time interval followed by a plateau with little to no changes in concentration following the initial period. This plateau is likely due to the exhaustion of O_3 presence in the reactor due to the significantly decreased stability of O_3 at high pH values (Beltran & Fernando, 2003). The data suggests that while the degradation rate constant is rapid initially, likely due to the presence of hydroxyl radicals, there is insufficient oxidant present to fully degrade the available OSP.

3.3.2.3 Matrix Effects on Oseltamivir Degradation

The effects of numerous cations and anions were monitored for their effects on the degradation capabilities of O_3 on OSP. The ions tested are found commonly in salts present in natural settings and monitored for in treatment plants allowing for testing concentrations to be generated via historical records. The anions tested were Cl^- (250 mg L^{-1}), NO_3^- (0.5 mg N L^{-1}), Br^- (2 mg L^{-1}) and SO_4^{2-} (100 mg L^{-1}) with concentrations chosen based on concentrations reports in wastewater treatment plants (Soltermann et al., 2016; Wu et al., 2010; Zhang et al., 2013). Among the anions tested, the apparent largest impactors were Cl^- and SO_4^{2-}, with Br^- and NO_3^- having relatively lower impact as can be seen in Figure 3.5. The cations tested were Na^+ (162.08 mg L^{-1}), K^+ (275.64 mg L^{-1}), Ca^{2+} (141.27 mg L^{-1}), Mg^{2+} (85.68 mg L^{-1}), and NH_4^+ (120.07 mg L^{-1}), with concentrations chosen to keep Cl^- concentration constant. All of the cations tested had similar effects on the degradation kinetics as seen in Figure 3.5. The data suggests that none of the ions have a statistically different effect on OSP degradation from a no matrix sample from an unpaired t-test shown in Table A.1; an ANOVA analysis shown in Table A.2 does

however state that there are statistically different differences within the population. The ionic strength of the solution does appears to have an impact on the apparent rate constant. This finding was tested by comparing the rate constants of two NaCl solutions of differing concentrations, which resulted in the 0.125 M NaCl solution with a rate constant (k') of 8.53×10^{-2} s^{-1} and the 0.250M NaCl with a k' = 7.47×10^{-2} s^{-1}. The impact of the ionic strength comes from its effect on the Henry's law constant (K_h) which has an effect on the solubility of ozone in water as described in Equation 6,

$$\ln K_h = aT^{-1} + b\mu + c\mu T^{-1} + d \qquad \text{Equation 6}$$

where $a - d$ are experimentally determined coefficients, T is the absolute temperature, and μ is the ionic strength (Kosak-Channing & Helz, 1983; Sotelo et al., 1989; Mekic et al., 2018). The ionic strength for each species can be found in Table A.2 where the ionic strength was calculated using Equation 7 where c is the molar concentration of the solution and z is the charge number of that ion. The decreased solubility of ozone in water may result in less available ozone for degradation, resulting in a decreased rate constant.

$$\mu = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2 \qquad \text{Equation 7}$$

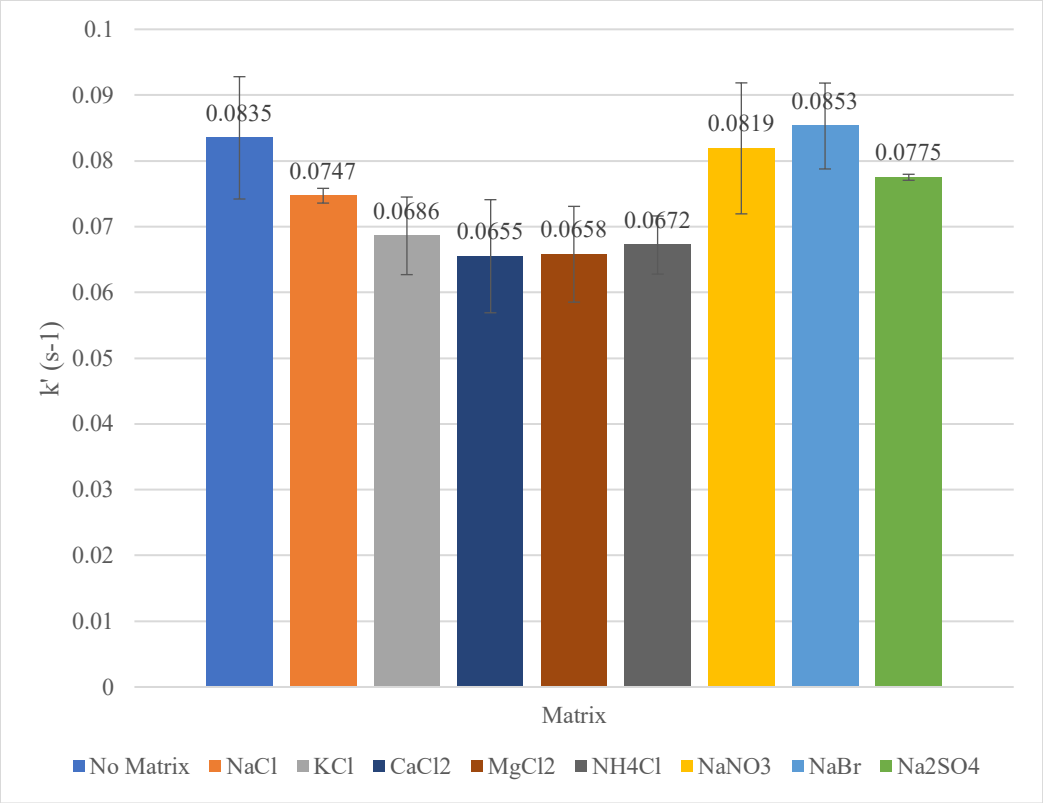

Figure 3. 5 Matrix effects of various anions and cations via ionic salts on the pseudo-first order degradation of OSP via O_3 batch treatment ([NaCl] = 7.05 mM, [KCl] = 7.05 mM, [$CaCl_2$] = 3.53 mM, [$MgCl_2$] = 3.53 mM, [NH_4Cl] = 7.05 mM, [$NaNO_3$] = 36.0 µM, [NaBr] = 25.0 µM, [Na_2SO_4] = 1.04 mM, [O_3] = 10 µM, reaction time = 30 s, Temp. = 21.7 – 22.4 ºC, pH = 6.9 – 7.1).

3.3.2.4 Oseltamivir Degradation in Wastewater

The effects of real wastewater matrixes on O_3 degradation of OSP were determined using UV-treated secondary effluent. The secondary effluent was characterized and found to contain a DOC of 7.249 mg L^{-1}, an ozone demand of 4.04 mg O_3 L^{-1}, Cl^- concentration of 232 mg L^{-1}, NO_3^- concentration of 9.81 mg L^{-1}, SO_4^{2-} concentration of 16.5 mg L^{-1}, and a Br^- concentration of <1 mg L^{-1}. By monitoring the concentrations of OSP, O_3, and the DOC levels, we are able to determine how the ozone is utilized in a real water system. Experimentally examining the removal oseltamivir with respect to the ratio of ozone to DOC (g O_3 / g DOC) allows us to determine how the system responds to varying conditions as well as determine the required

concentration to fully degrade the target compound. The ratios of g O_3 / g DOC ranged from 0.0 to 1.0 based on suggestions that pharmaceuticals that are either easily or moderately degradable by ozone require a ratio < 1.20 for a one-decade removal of pollutant (Antoniou et al., 2013). Figure 3.6 shows that oseltamivir degradation follows a linear relationship with increasing g O_3 / g DOC ratio, suggesting oseltamivir is broken down efficiently and early in the reaction simultaneously or before other organics from the DOC. The data shows that an ozone dose of 0.544 g O_3 / g DOC sufficiently removes >98% of residual OSP from the wastewater sample. The degradability of OSP via O_3 in wastewater is reinforced in the study by Ghosh et al. (2010) who found ozone effectively removed >90% of the oseltamivir metabolite oseltamivir carboxylate in a full-scale tertiary ozonation process. The monitoring of the ozone concentration shows that the available ozone is fully consumed during this process. As shown in Figure 3.6, there is an increase in DOC concentration as the ozone dose increases, this has been reported in other studies such as Papageorgiou et al. (2017) and Tregeur et al., (2010) who suggest this phenomenon results from ozone's ability to dissolve particulate organic carbon (POC), reducing its hydrophobicity and resulting in a DOC increase of the water.

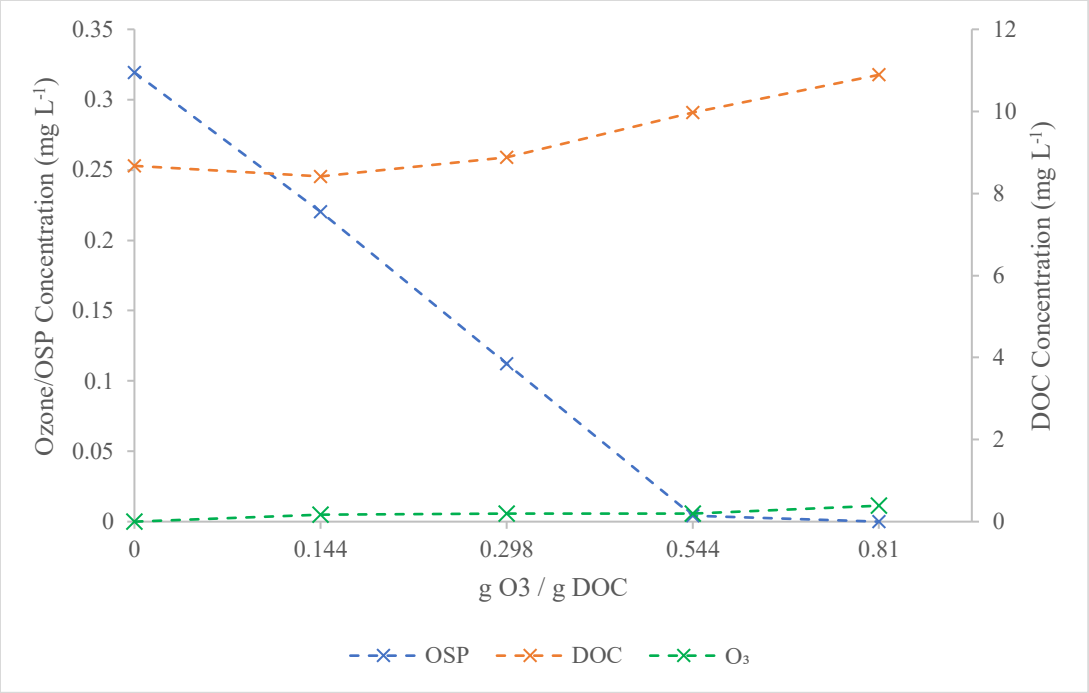

Figure 3. 6 Ozonation of OSP in secondary effluent showing changing concentrations of OSP, O_3, and DOC over time (OSP = 1.0 µM, O_3 = 0.0 – 5.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.15 mM, Temp. = 22.0°C, pH = 7.4 – 8.0, time = 60 seconds).

3.3.2.5 Molecular Ozone and Hydroxyl Radical Kinetics

Ozone degradation occurs via both direct and indirect reactions; direct reactions involve oxidation via the ozone molecule itself, and indirect reactions involve reaction with the hydroxyl radical, formed during the decomposition of ozone or from other direct ozone reactions (Beltran & Fernando, 2003). By monitoring the degradation via a singular reaction, it provides information on the most efficient mechanism for OSP degradation. Our studies examining the degradation of OSP via O_3 with the hydroxyl radical scavenger tert-butyl alcohol (TBA) are shown in Figure 3.7. At pH 7 found that the apparent rate constant only differed slightly, adjusting from 0.0807 s^{-1} without scavenger to 0.0721 s^{-1} with one. This experimental design repeated at pH 8.5 found similar results, with little difference between the scavenger-free rate constant of 0.113 s^{-1} and 0.119 s^{-1} with scavenger, suggesting that at neutral pH, direct oxidation

has the largest impact. When tested at a more basic pH of 10.0, the variation from the TBA increased, suggesting that hydroxyl radicals play a larger role in basic systems. This finding was expected due to ozone's lowered stability in basic environments leading to an increased formation of hydroxyl radical as outlined in section 3.2.2. While it is likely that hydroxyl radicals may serve to degrade OSP quicker due to their higher oxidation potential (E^o) of 2.72 V compared to ozone's potential of 1.89 V (Schwarz & Dodson, 1984), in our experiments the micropollutant is removed to a greater extent via molecular ozone. Based on the data shown in Figure 3.7, subtracting the molecular ozonation first-order rate constant (0.0721 s^{-1}) from the overall ozonation first-order rate constant (0.0835 s^{-1}) provides an estimated rate constant of 0.0114 s^{-1} with ·OH, note that a more accurate value can be obtained via a competitive experiment.

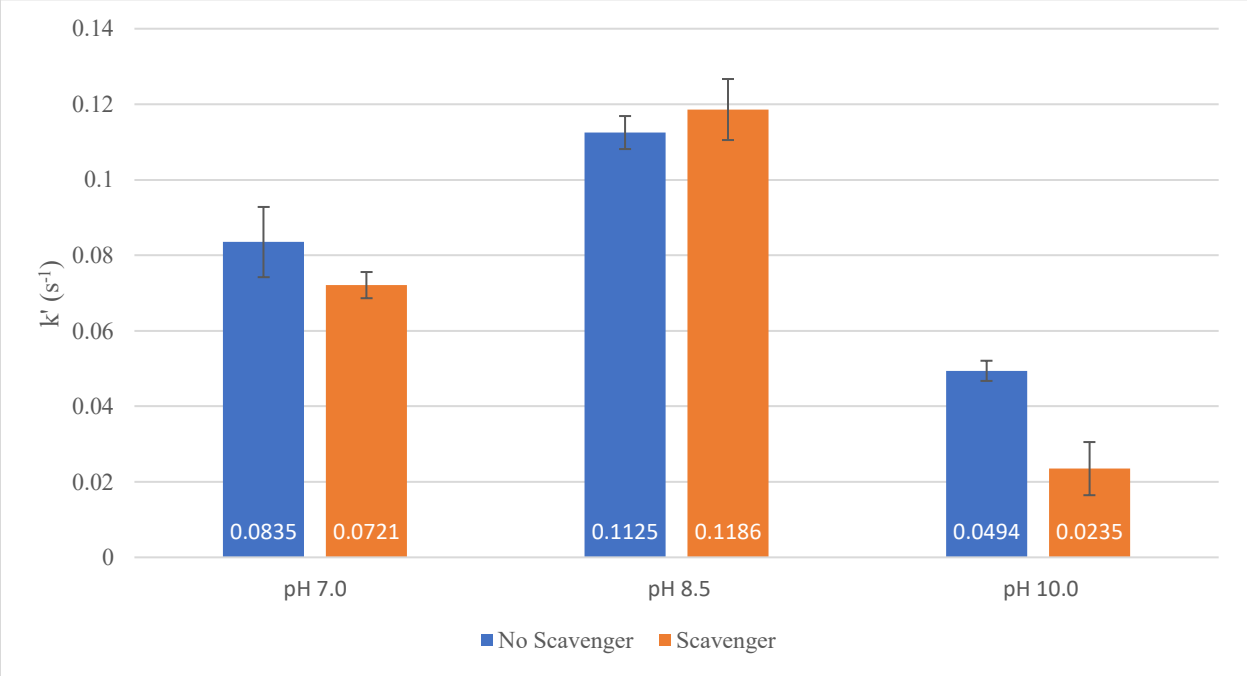

Figure 3. 7 Pseudo-first order rate constant values for OSP degradation with and without TBA scavenger at pH 7.0, 8.5 and 10.0 (OSP = 1.0 µM, O_3 = 10.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.15 mM, TBA = 100 µM, Temp. = 22.7 – 23.9°C, NaOH as pH adjustment).

3.3.3 Byproducts of Oseltamivir Degradation

The byproducts of the ozonation of OSP were determined in order to examine the significance of various compounds being produced during an ozone oxidation process. Studies have shown that the oxidation products produced from ozonation processes can result in increased toxicity with potential danger to aquatic organisms (Slater et al., 2010; Wu et al., 2019). Byproduct analysis performed here involved the use of HPLC-TOF-MS, making the elucidation of structures difficult without further testing, therefore structures suggested here are assumptions based on data suggested in other studies and predictions based on the structure and molecular formula of the parent compound (Gonçalves et al., 2011; Junwal et al., 2012; Wang et al., 2015). Proposed pathways for byproduct formation are shown in Figure 3.9.

Of the byproducts analyzed, some showed decreasing abundance with increased ozone exposure shown in figure 3.8. The products m/z 225, m/z 208, m/z 180, m/z 162, and m/z 166 all showed decreasing concentration over a longer ozone exposure period ranging from 0 – 30 seconds. The suggested structures and formulas of these products are shown in Figure 3.8. The decreasing concentration over time of these products suggest that they are further transformed or broken down into smaller constituents as ozone dose increases. Based on these findings, it would be less likely to find these transition products at high concentrations in natural water settings compared to more ozone resilient products.

Some of the degradation products showed increasing concentration correlating with increased ozone exposure as shown in Figure 3.8. The degradation products that followed this trend are m/z 327, m/z 345, m/z 361, and m/z 377, with structures and formulas shown in Figure 3.9. Byproducts m/z 345, m/z 361, and m/z 377 showed relatively low concentrations, with very small responses in the chromatograms, suggesting they are very minor products at most, the

exact conformation of these products is also not known. The byproduct with m/z 327 on the other hand had a very high relative response, with the largest integration value of all byproducts that were analyzed. The structure of m/z 327 is very similar to that of oseltamivir with Wang et al. (2015) suggesting that it is produced by a keto-derivatization. The relatively high abundance of this byproduct suggests that while oseltamivir itself is removed efficiently by ozonation, byproducts may be formed in significant quantities that have very similar structures to the parent compound.

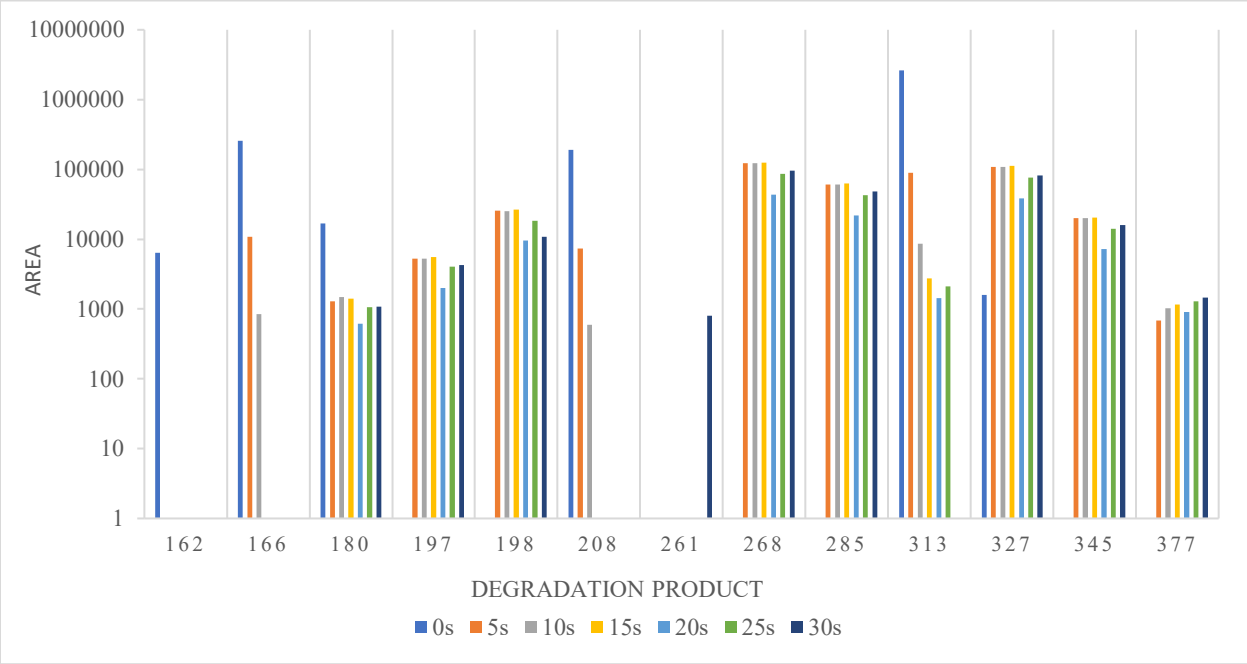

Figure 3. 8 Changing byproducts concentrations from ozonation of OSP with ozone exposure times ranging from 0 – 30 seconds. (OSP = 1.0 µM, O_3 = 10.0 µM, PO_4 buffer = 0.1 mM, $Na_2S_2O_3$ = 0.15 mM).

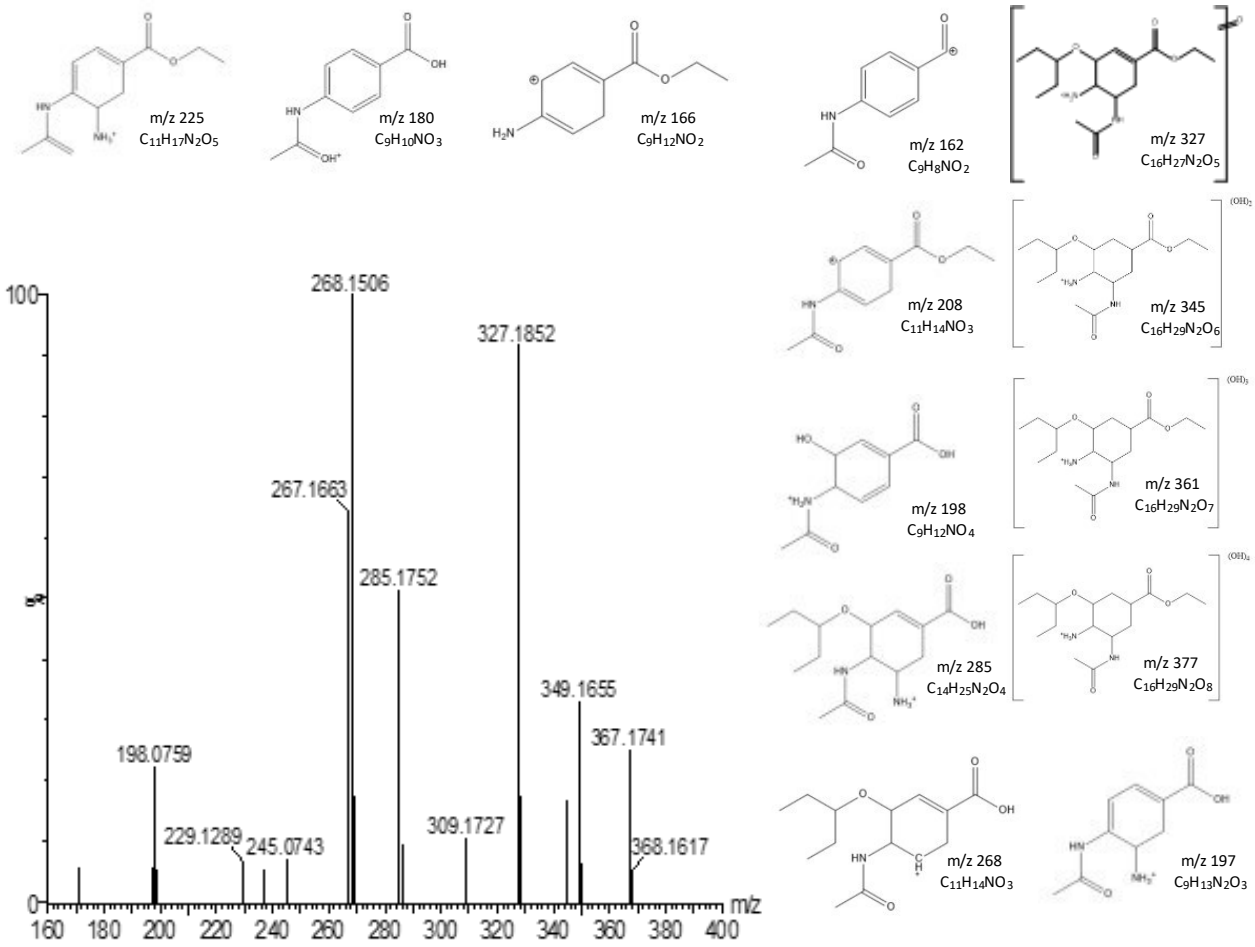

Figure 3. 9 Mass spectrum of ozonated OSP solution outlining byproducts of ozonation with structures and formulas of byproducts (byproduct structures suggested by Goncalves et al. (2011), Junwal et al. (2012), and Wang et al. (2015)). Byproducts with structures and formulas not reported are m/z 349, m/z 367, m/z 309, and m/z 245.

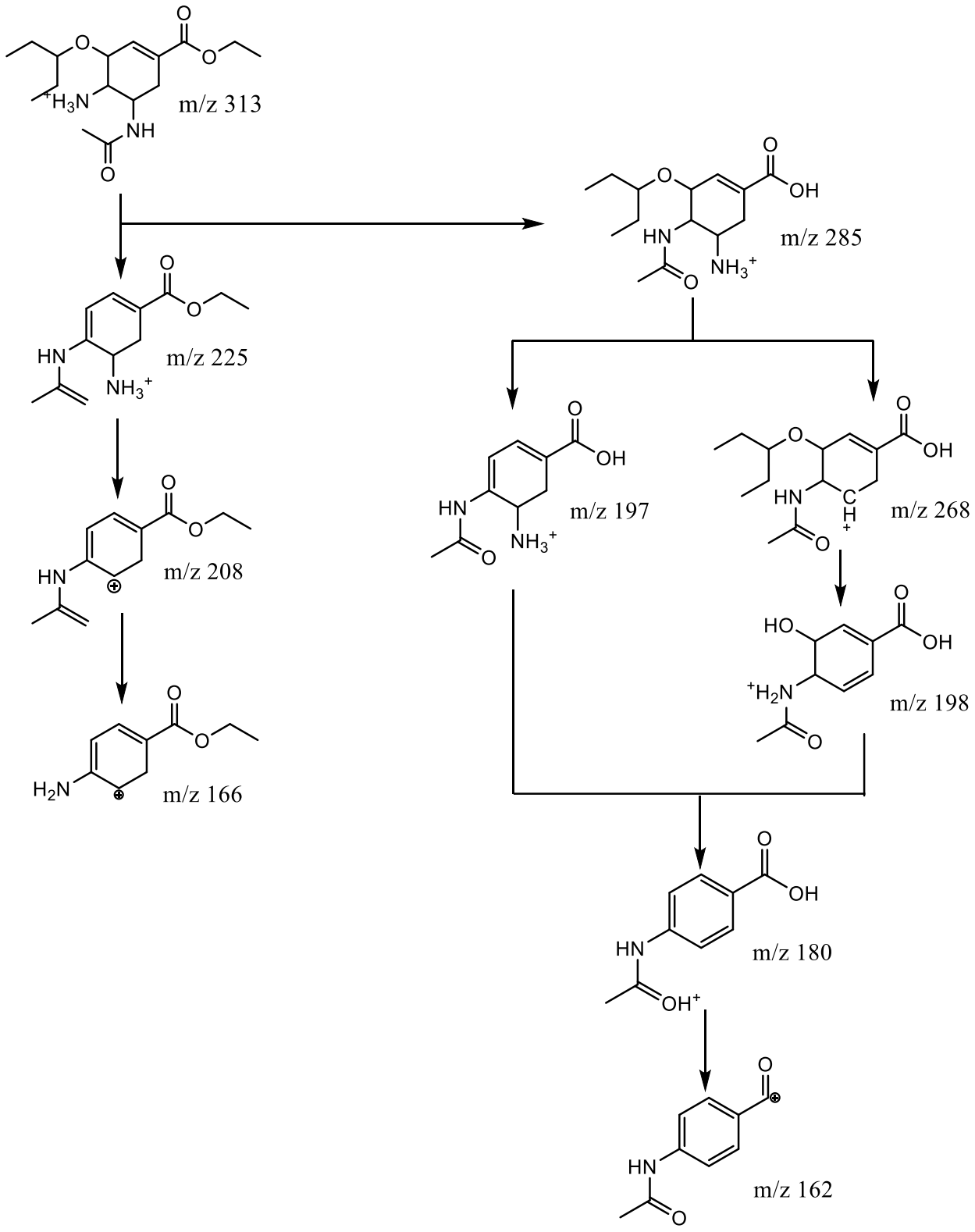

Figure 3. 10 Proposed pathway for the degradation of oseltamivir and production of degradation products via ozonation.

Other byproducts maintained a relatively high abundance with no clear change in concentration over ozonation period shown in figure 3.8. The products that fit into this category are m/z 285, m/z 268, m/z 198, and m/z 197 whose structures are shown in Figure 3.9. Byproducts represented in this section all follow the trend of having both relatively high concentrations and time of ozonation having no noticeable trend on their concentration, this suggests that these products are both produced in high concentrations and potentially resistant to ozonation processes. These findings suggest that the byproducts represented in this section have a higher likelihood of being discovered in natural water settings due to their abundance and resistance to degradation, two important factors related to environmental persistence (Webster, 1998).

Figure 3.10 outlines the proposed pathways of OSP degradation via ozonation. The proposed pathways were reported based on branching areas which can be more easily removed from the base structure and nucleophilic centres more susceptible to bond formation. The byproducts on the left pathway (m/z 225, 208, and 166) show decreasing concentrations over ozone exposure suggesting they likely have low energy barriers for degradation and further breakdown into constituents. Products shown on the right pathway (m/z 197, 198, 268, and 285) are present in larger concentrations and appear to be more persistent to ozone degradation, suggesting they have greater energy barriers of degradation.

3.3.4 Toxicity Analysis

3.3.4.1 Acute Toxicity Analysis via Bioluminescence Inhibition Test (*V. fischeri*)

The acute toxicity of oseltamivir and byproducts was monitored for acute toxicity against *V. fischeri* to determine the potential negative effects of oseltamivir and ozonation of oseltamivir.

Samples were set up to provide information on oseltamivir toxicity in both pure water and real wastewater matrices for both the parent compound and any potential oxidation byproducts produced during ozonation. The oseltamivir concentration examined in these experiments was set to 1.0 μM (0.41 mg L^{-1}) with the goal of being near to the strongest reported concentration in surface waters of 556.9 ng L^{-1} which was reported in Japan (Takanami et al., 2012; Soderstrom et al., 2009; Jarhult et al., 2011) Results in Figure 3.11 show that oseltamivir produces no positive acute toxicity towards *V. fischeri* in either water or wastewater.

Toxicity experiments also examined the post-ozonation solutions in order to determine the possibility of production of toxic intermediates or disinfection byproducts. Reasoning for this analysis comes from a study of the oxidation of the antiviral drug acyclovir which produced N-(4-carbamoyl-2-imino-5-oxoimidazolidin)-formamido-N-methoxyacetic acid (COFA) which was found to have acute bacterial toxicity (Prasse et al., 2012). As shown in Figure 3.11, no increase in acute toxicity was observed from ozonation of oseltamivir in ODFW or secondary effluent matrices, suggesting the products do not increase bacterial toxicity.

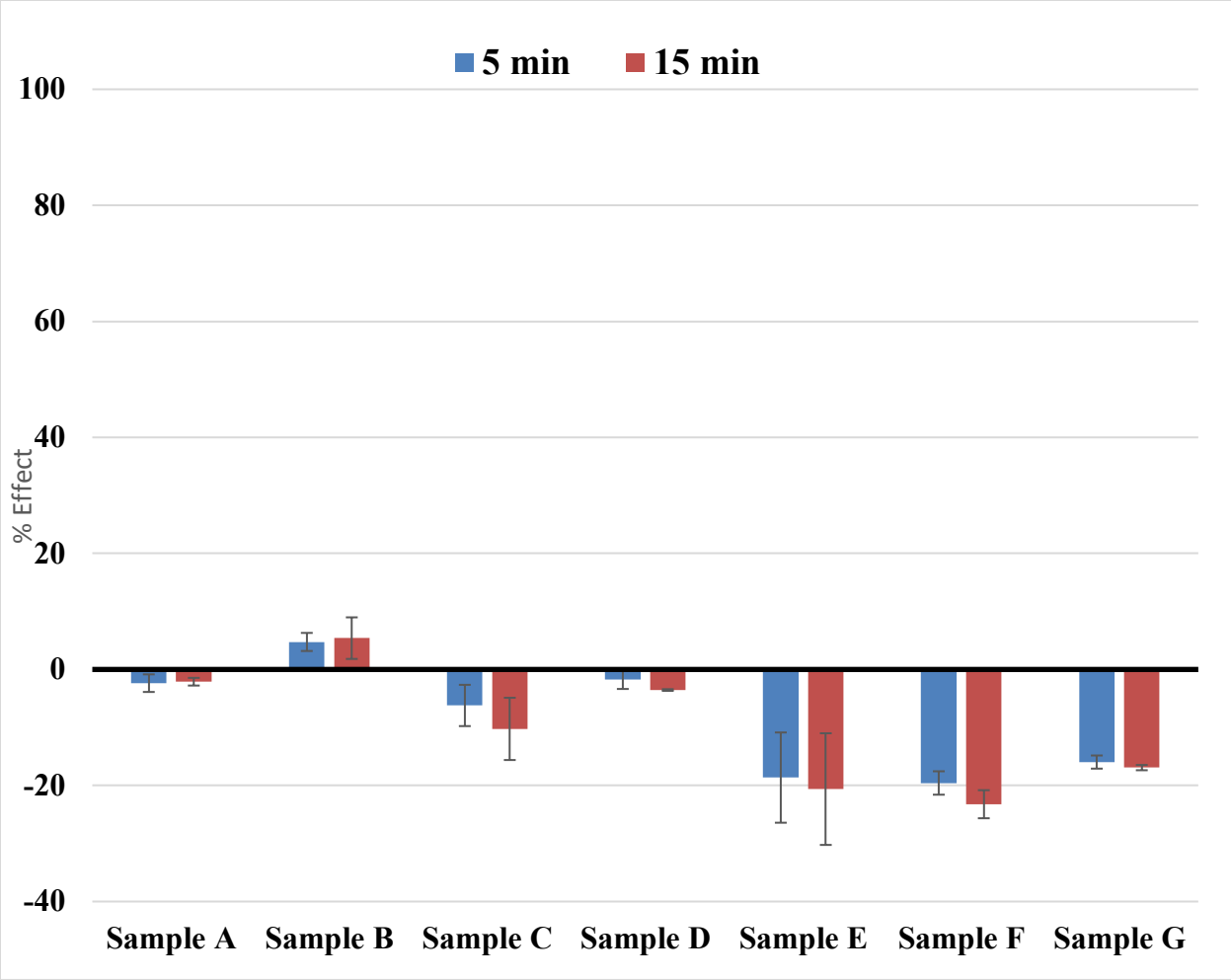

Figure 3. 11 Percent inhibition of acute toxicity samples A) Pure ODFW, B) Buffered ODFW with sodium thiosulfate, C) OSP control, D) ozonated OSP sample, E) Secondary effluent, F) Ozonated secondary effluent, G) OSP spiked ozonated secondary effluent (negative effect indicates no acute toxicity).

3.3.4.2 Genotoxicity in Escherichia Coli (Mutagenicity)

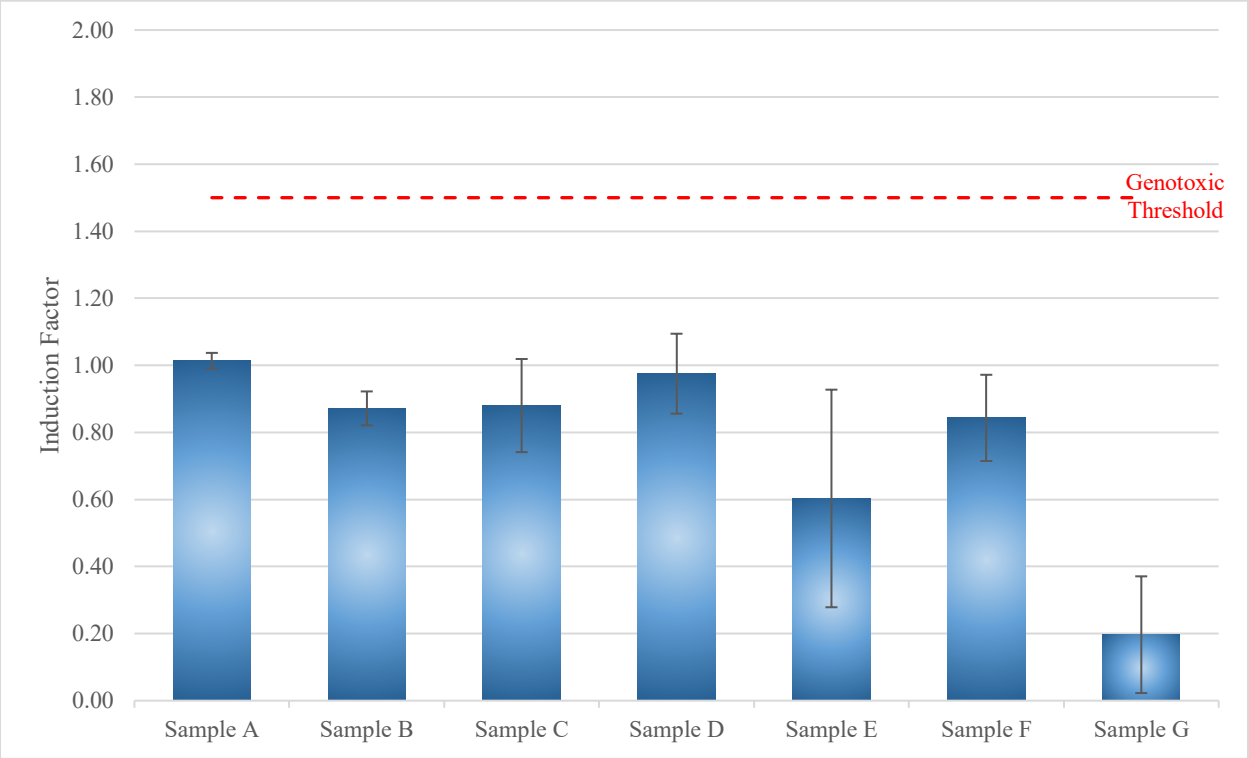

Figure 3. 12 Induction factors of genotoxicity samples A) Pure ODFW, B) Buffered ODFW with sodium thiosulfate, C) OSP control, D) ozonated OSP sample, E) Secondary effluent, F) Ozonated secondary effluent, G) OSP spiked ozonated secondary effluent.

Genotoxicity analysis was performed very similarly to the acute bacterial toxicity tests in section 3.4.1 with emphasis examining OSP in ODFW and secondary effluent matrices both before and after ozone treatment. The SOS-ChromoTest used here is a replacement for the Ames test for genotoxicity measurement, it is a colorimetric assay which measures expression of genes caused by genotoxic agents via a fusion with the structural gene for β-galactosidase (Quillardet & Hofnung, 1993). Results from the genotoxicity test are shown in Figure 3.12. The survival rate for all samples utilized was >80%, according to the manufacturers specifications, this indicates the samples are valid and not cytotoxic to the test bacteria. All reported IFs were less than a value of 1.1, significantly lower than the manufacturers genotoxic threshold of 1.5. This suggests that all samples tested were not genotoxic and did not contain genotoxic substances.

Interestingly, sample G which included ozonated secondary effluent spiked with OSP had a lower IF than other samples, suggesting the addition of OSP and oxidation byproducts may have decreased the genotoxicity of the effluent. These findings are in line with those discussed by Ila & Ilhan (2012) who found no evidence of genotoxic dose-dependency in sister chromatid exchange, chromosomal aberration, and cytokinesis-blocked micronucleus assays.

3.4 Conclusion

This study showed that ozonation as a tertiary treatment for the removal of oseltamivir is a strong option for removal where traditional treatment methods have failed. The effect of matrix pH on the degradation process was found to have some strongly negative effects on the extreme high-end of the spectrum, but in all other regions remains an effective option for the removal of oseltamivir. Matrix effects from various ions were also shown to have minimal effect on ozone degradation, with the largest effect likely coming from the ionic strength of the matrix. The degradation of OSP in wastewater proved to be effective, with a 0.544 g O_3/g DOC ratio capable of removing >95% of present OSP. Many of the most abundant byproducts of OSP ozonation were identified and pathways of formation were suggested, these byproducts were also shown to produce no acute toxicity towards *V. fischeri* and were not found to cause any increase in potential genotoxicity.

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Chapter 4: Conclusions and Recommendations

4.1 Thesis Overview

Antiviral drugs can be characterized by their diversity in structure, toxicological effects, environmental fate, and their ability to be removed in conventional treatment methods. The release of antiviral drugs is a threat to the stability and health of ecosystems; while the removal of these drugs can prove to be difficult, it is vital that we work towards a situation where we minimize our impact on local environs. Primary and secondary wastewater treatment processes such as screening, sedimentation and biological processes remain a valuable resource for removing suspended and dissolved matter. Despite the significant benefits of primary and secondary treatments, they fail to effectively remove many organic compounds, thus creates the requirement for oxidative processes designed for the disinfection and removal of organic compounds. Among those disinfection techniques is ozone which has shown promise in the removal of many traditionally persistent compounds due to its strong oxidative properties. Significant research has gone into the application and use of ozone as a wastewater treatment technique, however the byproducts generated after treatment remain unknown.

The second chapter of this thesis discusses research conducted in the realm of wastewater treatment in relation to antiviral drugs. The types of antiviral drugs and what health risks are associated is a vital piece of information to understand the big picture and provide suggestions on how unknown antiviral drugs may affect local ecosystems. The detection methods many for antiviral drugs in both natural water and wastewater settings has been determined, allowing for examination of the removal efficiencies of various forms of treatment processes and full-scale treatment plants. Despite the research that has occurred looking at treatment processes, there are many antivirals for which information is severely lacking. The environmental fate of antivirals is

a complex process that involves numerous factors including photodegradation, biodegradation, and adsorption which is largely unknown for many compounds. Due to the lack of information around the environmental fate of antiviral drugs, a resulting gap in the knowledge surrounding the potential toxicological effects presents itself. Very few studies have been conducted examining the long- or short-term effects of antiviral drugs on aquatic organisms, leading to misunderstandings around the severity and importance of their removal.

The third chapter discusses the treatment of oseltamivir phosphate via ozone oxidation and the factors that affect that process. It was found that ozonation was a capable method for the degradation of ozone, in pure water, the degradation was discovered to follow second order kinetic rules and ozone was able to >95% of oseltamivir present within 30 seconds. The factors that affect degradation can include pH, which can change throughout the year in wastewater; moving pH away from the neutral range was found to decrease degradation rate when approaching both acidic and basic extremes. The effect of the water matrix and ions present within was also investigated and was found to have minimal effect the ozone capabilities. An important area related to water oxidation is the production of byproducts due to their unknown and potentially dangerous nature; for oseltamivir, byproducts of ozonation were identified and tested for acute toxicity towards *V. fischeri* and genotoxicity which both came back negative.

4.2 Conclusions

In summary, the research presented in this thesis focussed on the use of advanced oxidation in the form of ozone with the purpose of removing oseltamivir from wastewater. The literature review presented within outlined the gaps in current knowledge related to the wastewater treatment of antiviral drugs, these gaps in knowledge provided the direction for the research project discussed within. The emphasis on this research was ozonation as a treatment

process through understanding the underlying mechanisms and outcomes related to this promising technique.

The removal of oseltamivir phosphate using ozonation was shown to be a very promising technique. The removal of oseltamivir from wastewater showed that a removal rate of >98% could be achieved with an ozone dosage of 0.544 g O_3/g DOC in 60 seconds. The kinetics of the reaction between oseltamivir and ozone follow the second order rate law and were found to be between 6787 – 9206 $M^{-1}s^{-1}$ using two different methods of calculation. The effects of various ions including Cl^-, Br^- SO_4^{2-}, NO_3^-, Na^+, Ca^{2+}, Mg^{2+}, K^+, and NH_4^+ on the rate of degradation were measured and found that none of which had a significant impact, but the ionic strength of the water likely has some negative effect. At neutral pH, the primary mechanism of oxidation via ozone was through molecular ozone at neutral pH with a shift towards hydroxyl radicals as the pH increased and molecular ozone lifespan decreased. The acute toxicity towards *V. fischeri* and genotoxic effects of oseltamivir and its DBPs were measured, and it was found that none of which caused any increase in toxicity.

4.3 Recommendations

The research presented herewith may lay groundwork for future research endeavours. The following are propositions for work to be conducted in future research based on gaps found in current and previous studies.

- Bench- or full-scale studies examining the removal of antiviral drugs via aerobic and anerobic digestion processes. Throughout my examination of literature, there were few studies found that specifically examined these processes, and instead examined wastewater treatment operations including the entire process train. Further examination into the conditions that allow these processes to thrive and information

on which antivirals are susceptible to this treatment method will provide more thorough understanding on the mechanisms, kinetics, and contributing factors that affect the process.

- Research examining the toxicity of degradation products resulting from advanced oxidation processes applied to the antiviral drugs. Few studies have focussed on the degradation products resulting from wastewater treatment, the possibility of generating potentially more toxic compounds than the parent compound exists and has been shown in studies related to the antiviral acyclovir. By looking at antiviral drugs individually, researchers will be able to screen for increases in various types of toxicity that may result from the degradation of the parent compounds.
- Photocatalytic advanced oxidation processes for the treatment of antiviral drugs. Studies have shown photocatalytic processes to be significantly more effective against antivirals than UV treatment alone. Further research into the types of catalysts may allow to produce cost effective and efficient tools for wastewater disinfection.
- Studies examining the environmental fate of antiviral drugs once they are released from treatment plants. Investigating how antiviral drugs behave once they are introduced into natural waters will provide information on how and where they accumulate. Pollutants can accumulate in wildlife, plants, lakes, rivers, and soil; understanding the endpoint of pollution will provide knowledge on which antivirals pose the most significant risk.
- Examination of the effects of wastewater conditions on the production of degradation products from oseltamivir ozonation. The generation of byproducts has the potential to change based on the conditions of the degradation, studying this process in real

water and wastewater matrixes may result in different, potentially dangerous products that should be identified.

- Examination of the use of catalytic ozonation and its effectiveness on oseltamivir and other antiviral drugs. Various catalysts made from carbon, metals, or other materials can improve upon some problems such as mineralization and toxic byproduct production. Studies examining the use of iron, manganese, or carbon nanoparticle-based catalysts may prove to improve upon techniques.

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Appendix

Supplementary Figures

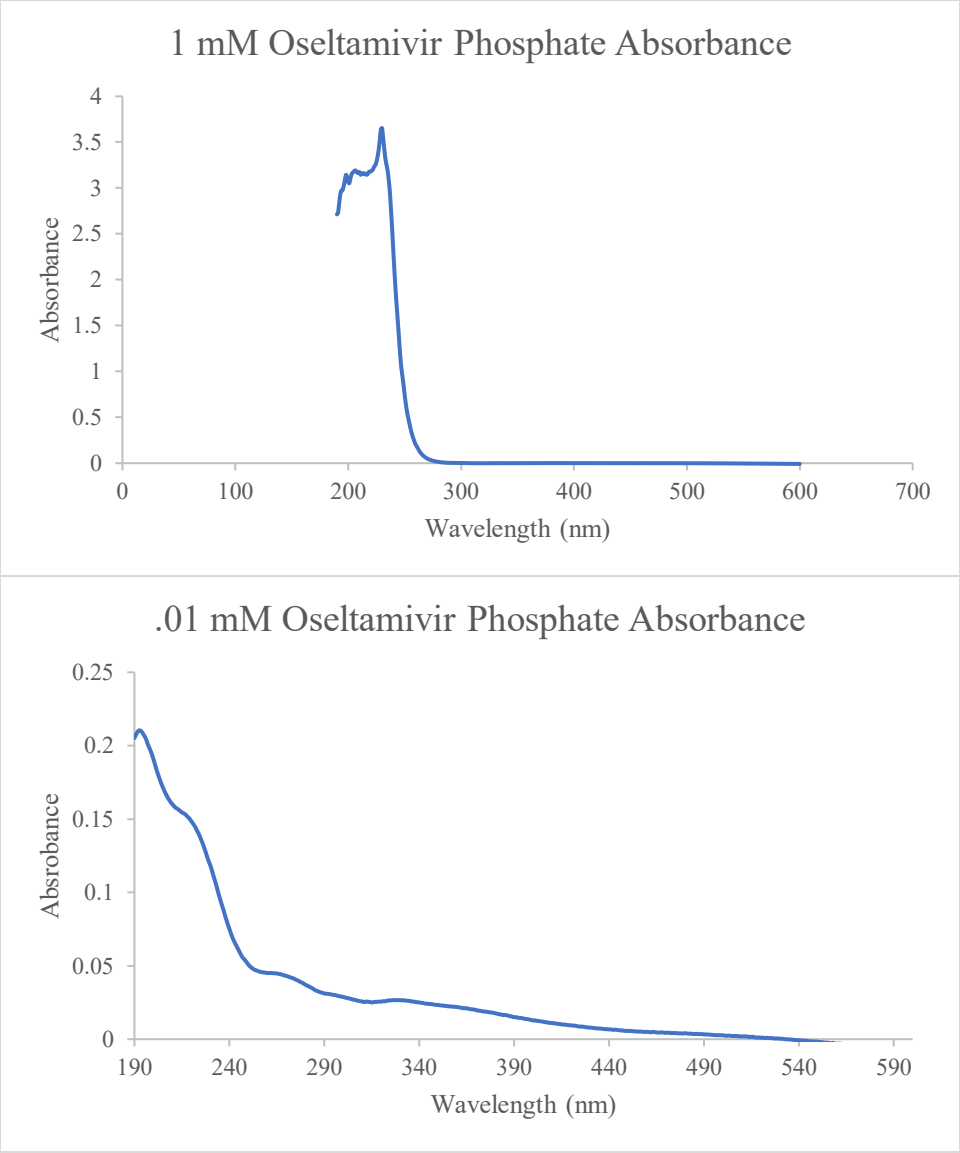
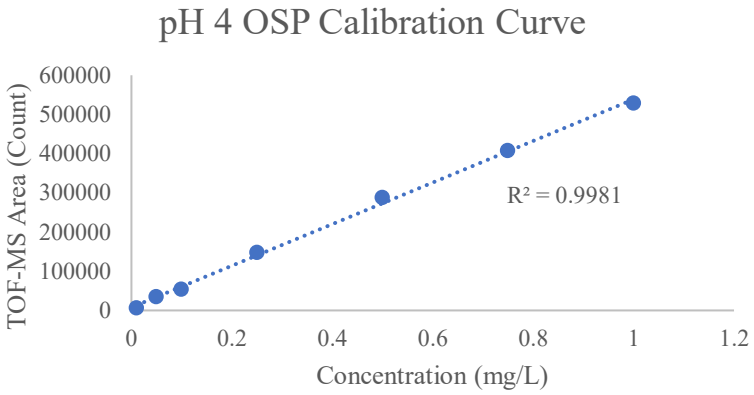
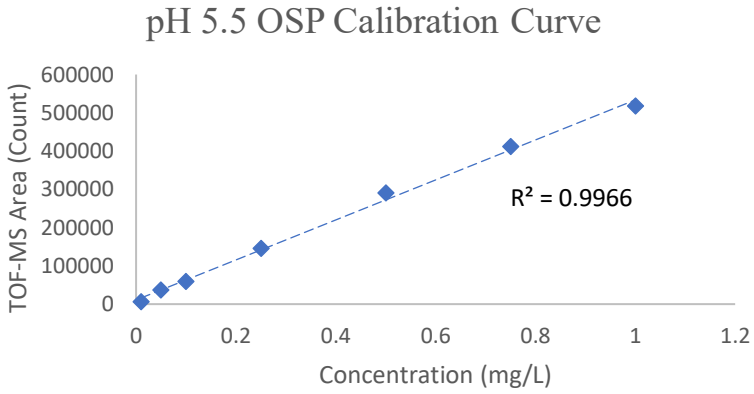


Figure A.1 Absorbance spectra of oseltamivir phosphate


pH 4 OSP Calibration Curve
TOF-MS Area (Count)
600000
500000
400000
300000
200000
100000
0
R² = 0.9981
0
0.2
0.4
0.6
0.8
1
1.2
Concentration (mg/L)


pH 5.5 OSP Calibration Curve
TOF-MS Area (Count)
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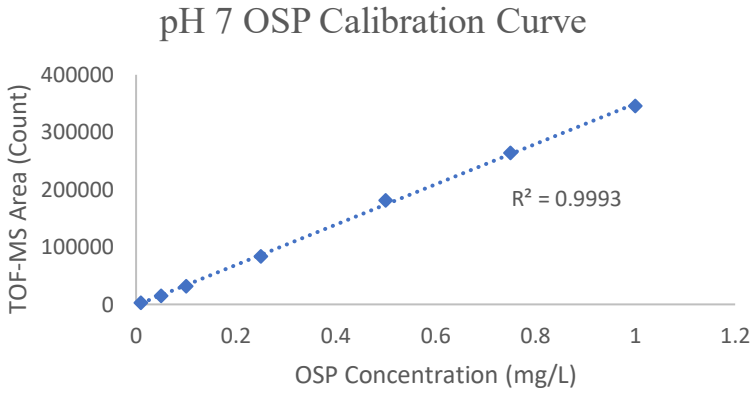
500000
400000
300000
200000
100000
0
R² = 0.9966
0
0.2
0.4
0.6
0.8
1
1.2
Concentration (mg/L)


pH 7 OSP Calibration Curve
TOF-MS Area (Count)
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300000
200000
100000
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R² = 0.9993
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0.2
0.4
0.6
0.8
1
1.2
OSP Concentration (mg/L)

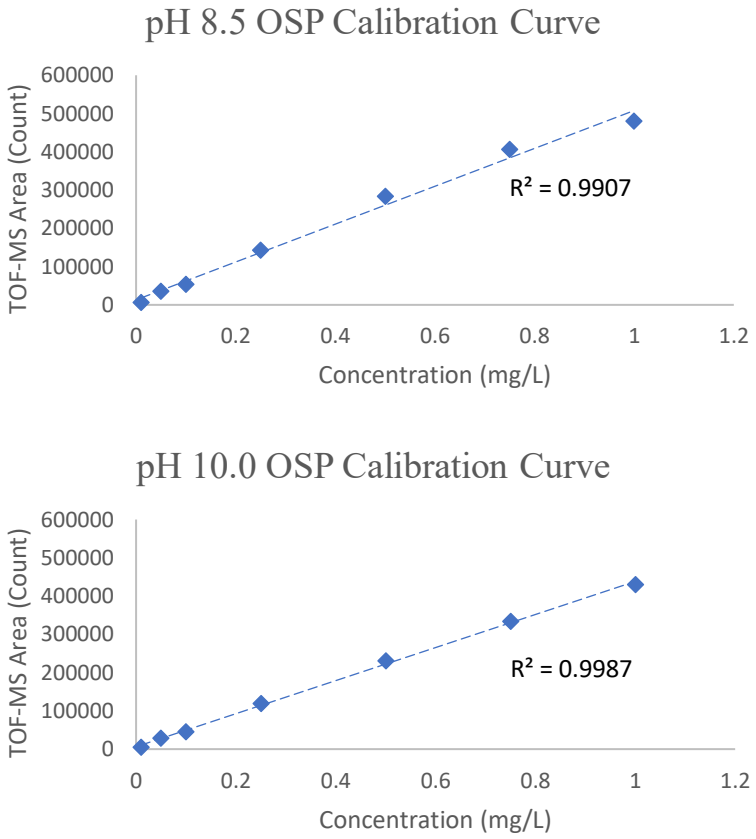

Figure A.2 Mass spectrometry calibration curves for oseltamivir phosphate at multiple pH values.

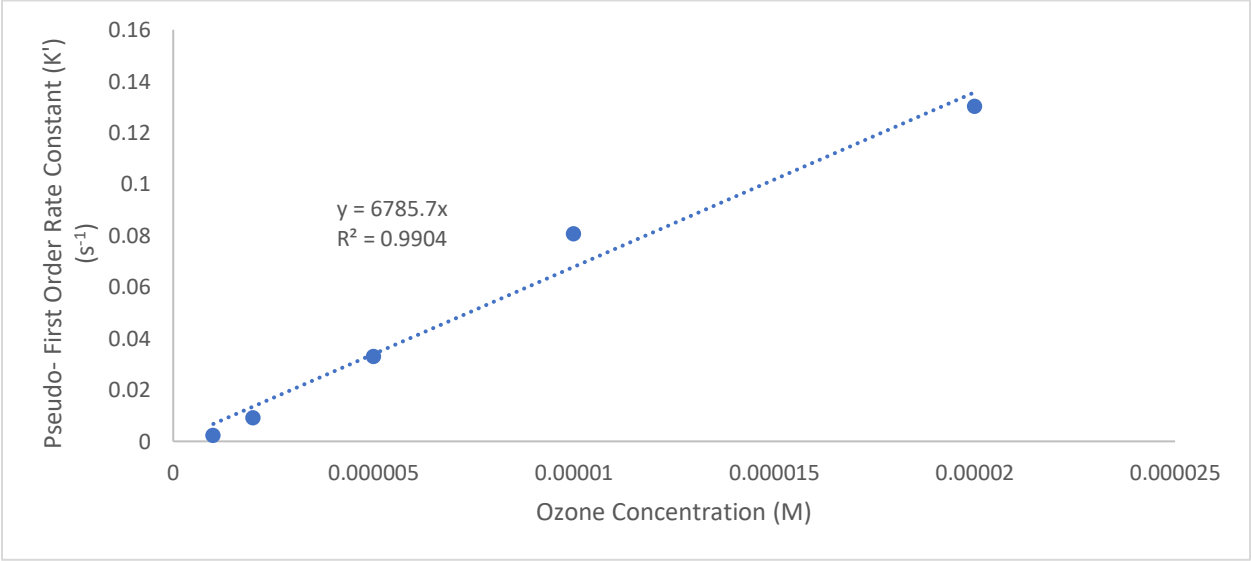

Figure A.3 Plot of pseudo first-order rate constant against ozone concentration ti determine of second-order rate constant.

Table A.1 95% confidence unpaired t-test results of matrix effects for mean differences (Mean: average first order rate constant. S.D.: standard deviation of rate constant, N: number of samples run, P-Value: Probability the difference is by chance, C.I.: 95% confidence interval).

	No Matrix	NaCl	KCl	$NaNO_3$	NaBr	Na_2SO_4	$CaCl_2$	$MgCl_2$	NH_4Cl
Mean	0.0835	0.0747	0.0686	0.0819	0.0853	0.0775	0.0655	0.0658	0.0672
S.D.	0.0093	0.00112	0.0059	0.00996	0.00653	0.00046	0.0086	0.00728	0.00442
N	3	3	3	3	3	3	3	3	3
P-Value	N/A	0.179	0.0791	0.849	0.797	0.327	0.0696	0.0603	0.0518
C.I.	N/A	-.0062154 to 0.0238154	-.002755 to .032555	-.0202436 to .0234436	-.0200156 to .0164156	-.0089260 to .020926	-.002305 to .038305	-.0012320 to .0366320	-.0002058 to .0328058

Table A.2 Single Factor ANOVA analysis of ion species effects on degradation of oseltamivir phosphate

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
No Matrix	3	-0.2547172	-0.08490573	1.35054E-05
NaCl	3	-0.20498857	-0.06832952	1.24123E-05
KCl	3	-0.20724636	-0.06908212	2.76333E-05
$NaNO_3$	3	-0.24749697	-0.08249899	1.02104E-05
NaBr	3	-0.25542897	-0.08514299	2.77726E-05
Na_2SO_4	3	-0.23272093	-0.07757364	3.21629E-06
$CaCl_2$	3	-0.19982281	-0.0666076	3.50209E-05
$MgCl_2$	3	-0.20356575	-0.06785525	4.51414E-05
NH_4Cl	3	-0.19995886	-0.06665295	1.52638E-05

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00159015	8	0.000198769	9.406619318	4.62253E-05	2.510157895
Within Groups	0.000380353	18	2.11307E-05			
Total	0.001970503	26				

Table A.3 Ionic strength calculations for solutions used in experiments to determine matrix effects.

	Conc. (mM)	Ion 1 Count	Ion 1 Charge	Ion 2 Count	Ion 2 Charge	Ionic Strength (mM)
Na_2SO_4	1.04	2	1	1	2	6.24
NaBr	0.025	1	1	1	1	0.05
$NaNO_3$	0.036	1	1	1	1	0.072
KCl	7.05	1	1	1	1	14.1
NaCl	7.05	1	1	1	1	14.1
$CaCl_2$	3.525	1	2	2	1	21.15
$MgCl_2$	3.525	1	2	2	1	21.15
NH_4Cl	7.05	1	1	1	1	14.1

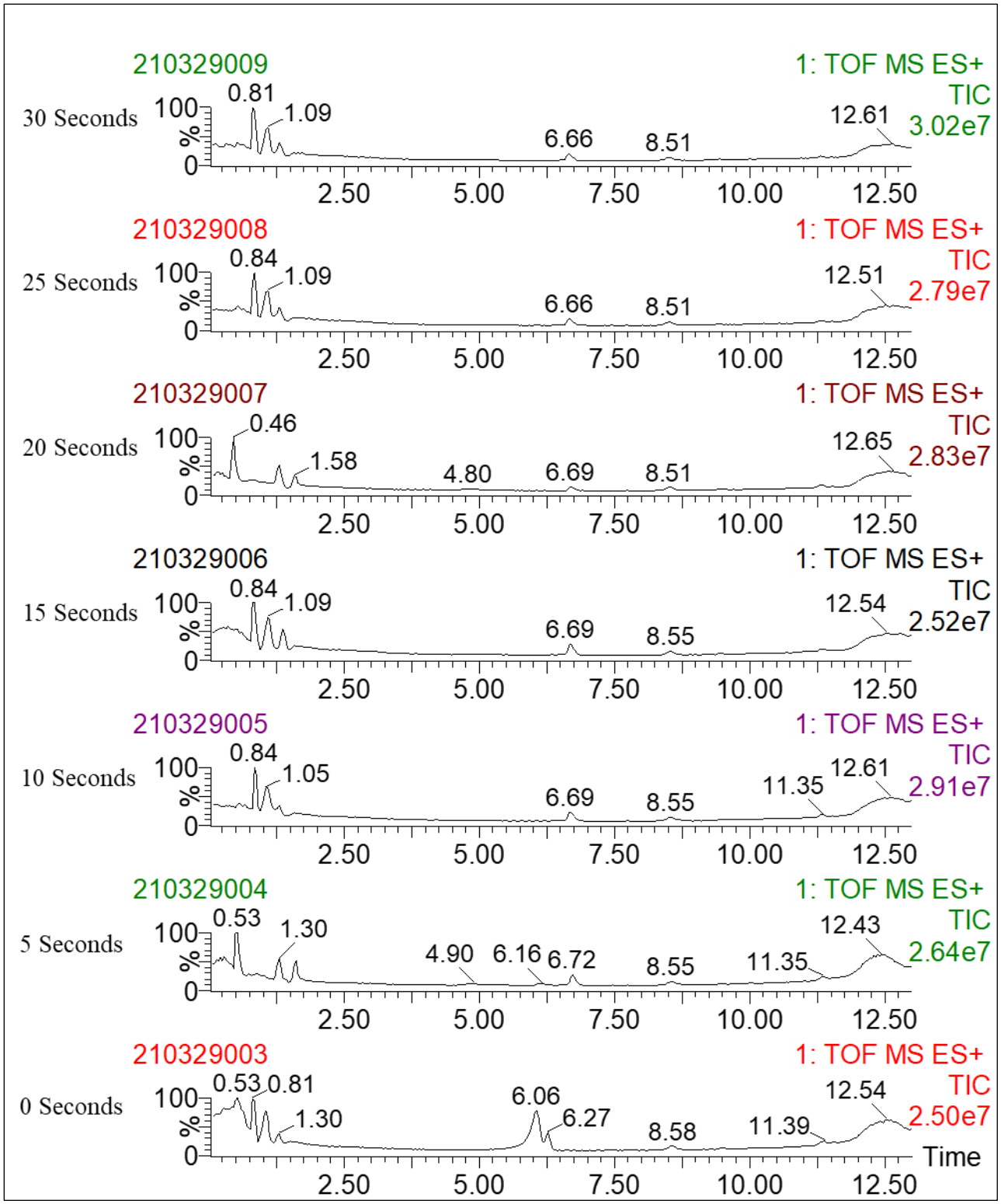

Figure A.4 Mass chromatograms for timed ozonation samples of oseltamivir phosphate.